University of Alberta

Epinephrine, Hypovolemia and the Neonate

by

David Lee Bigam ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

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in

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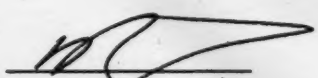

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Abstract

The effect of continuous epinephrine infusion on renal and mesenteric blood flow was studied in both normovolemic and hypovolemic piglets.

Superior mesenteric artery (SMA) and left renal artery ultrasonic flow probes were inserted into 16, 1-3 day old piglets. Two days later, the effects of epinephrine on SMA and renal blood flow, mean arterial pressure (MAP) and central venous pressure were measured. Randomized epinephrine doses of 0.2, 0.4, 0.8, 1.6 and 3.2 μg/kg/min were used in conscious, non-sedated piglets. Normovolemic piglets were subsequently bled (20 ml/kg) and received the same epinephrine infusion.

Significant increases in SMA and renal vascular resistance and MAP and decreases in SMA and renal blood flow occurred only with high dose epinephrine. Low to moderate dose epinephrine caused no significant decrease in SMA or renal blood flow and in fact was associated with an increase in blood flow in the hypovolemic piglets.

Low dose epinephrine may act as a renal and mesenteric vasodilator in the hypovolemic setting. Low to moderate dose epinephrine should not cause renal or mesenteric ischemia in the normovolemic or hypovolemic neonate.

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Table of Contents

Chapter 1

Introduction..... 1

Chapter 2

Epinephrine and Adrenoceptors..... 4

Chapter 3

The critically ill neonate..... 21

Chapter 4

The use of a chronic model..... 26

Chapter 5

The use of a porcine model..... 28

Chapter 6

The hypovolemic model..... 32

Chapter 7

Summary..... 37

Chapter 8

Materials and Methods..... 38

Surgical procedure..... 38

Experimental procedure..... 40

Chapter 9

Results..... 44

Normovolemia and Epinephrine..... 44

Hypovolemia and Epinephrine..... 45

Hypovolemia, no epinephrine..... 46

Tables..... 49

Figures..... 52

Chapter 10

Discussion..... 67

Chapter 11

Conclusion..... 82

Bibliography..... 84

List of Tables

page

Table 1: **Baseline Values**..... 49

Table 2: **Absolute values**..... 50

Table 3: **Percentage change from baseline**..... 51

List of Figures

page

Figure 1: Effects of Epinephrine on Mean Arterial Pressure - Normovolemia.....52

Figure 2: Effects of Epinephrine on Superior Mesenteric Artery Blood Flow - Normovolemia.....53

Figure 3: Effects of Epinephrine on Superior Mesenteric Artery Vascular Resistance - Normovolemia.....54

Figure 4: Effects of Epinephrine on Renal Blood Flow - Normovolemia.....55

Figure 5: Effects of Epinephrine on Renal Vascular Resistance - Normovolemia...56

Figure 6: Effects of Epinephrine on Mean Arterial Pressure - Hypovolemia.....57

Figure 7: Effects of Epinephrine on Superior Mesenteric Artery Blood Flow - Hypovolemia.....58

Figure 8: Effects of Epinephrine on Superior Mesenteric Artery Vascular Resistance - Hypovolemia.....59

Figure 9: Effects of Epinephrine on Renal Blood Flow - Hypovolemia.....60

Figure 10: Effects of Epinephrine on Renal Vascular Resistance - Hypovolemia....61

Figure 11: Effects of Hypovolemia on Mean Arterial Pressure.....62

Figure 12: Effects of Hypovolemia on Superior Mesenteric Artery Blood Flow.....63

Figure 13: Effects of Hypovolemia on Superior Mesenteric Artery Vascular Resistance.....64

Figure 14: Effects of Hypovolemia on Renal Blood Flow.....65

Figure 15: Effects of Hypovolemia on Renal Vascular Resistance.....66

List of Abbreviations

ARF - acute renal failure

BP - blood pressure

cAMP - cyclic adenosine monophosphate

CO - cardiac output

CVP - central venous pressure

GI - gastrointestinal

HR - heart rate

MAP - mean arterial pressure

NEC - necrotizing enterocolitis

PAP - pulmonary arterial pressure

PPH - persistent pulmonary hypertension

PRU - peripheral resistance units

PVR - pulmonary vascular resistance

RBF - renal blood flow

RVR - renal vascular resistance

SMA - superior mesenteric artery

SMABF - superior mesenteric artery blood flow

SMAVR - superior mesenteric artery vascular resistance

SV - stroke volume

SVR - systemic vascular resistance

Chapter 1

Introduction

Care of the critically ill neonate has improved dramatically over the last several years. There are however still many questions to be answered. What should be the first choice for an inotrope? What can be done to prevent necrotizing enterocolitis (NEC)? Does the newborn respond to hypovolemia in the same manner as an adult? These are a few of the questions addressed in this study.

The use of inotropes in neonates has been generalized from use in adults. However there are many factors that may make significant differences in response to inotropes between these age groups. Several studies have looked at adrenergic receptor ontogeny and how this could lead to an altered response to inotropes in the neonate (31-37,57,64,65,75,101). There is also mounting evidence for significant differences in the stress response in neonates as compared to adults (31,84,137).

Dopamine has traditionally been the first choice for an inotrope in the neonate however there is evidence that dopamine should be used with caution in this age group (14,52,80,137). Potential problems with dopamine in the newborn include a worsening of pulmonary hypertension and a lack of selective renal vasodilation (14,107,108).

Epinephrine was chosen for study for several reasons. Despite its widespread clinical use, studies of epinephrine use in the newborn population are quite limited to date. Epinephrine given as a bolus dose is used in cardiopulmonary resuscitation and for anaphylaxis in both neonates and adults (1,86,113). Epinephrine as a continuous

infusion has been used in critically ill patients, both adult and neonate, for years however it is usually used as a second line inotrope because of fears of renal or mesenteric ischemia (44, 116). Epinephrine is thought to increase cerebral and coronary blood flow at the expense of other (eg. renal or mesenteric) regional blood flows during times of stress (1). However the extent of this decrease in regional blood flow has not been well documented in the neonatal population. One previous study involving epinephrine infusion in the neonate actually revealed an increase in renal blood flow with a decrease in renal vascular resistance at varied dosages (12). The effects of a continuous, intravenous epinephrine infusion are examined in this study.

In 1966, Coffin et al studied the effects of epinephrine when it was used to treat low cardiac output (44). They showed that doses up to 1.0 μg/kg/min produce primarily a cardiotonic response, with an increase in peripheral and hepatosplanchnic blood flow, without significant peripheral vasoconstriction. With doses of 1.5 μg/kg/min and greater, a dominant peripheral vasoconstriction effect was observed with decreased peripheral return and cardiac output. The authors concluded from their experiments and from clinical use that continuous epinephrine infusion of 0.1-0.5 μg/kg/min was effective in the treatment of postoperative low cardiac output syndrome. Despite these results epinephrine has remained a second line inotrope in the care of the critically ill.

There have been several studies of regional blood flow in the acute setting, both with and without exogenous catecholamines, using perivascular flow probes or

the microsphere technique (12,31-37,54,64). Since anesthesia effects can be difficult to control (5,10,15,25,88,132), a chronic model involving a piglet that is conscious and non-sedated may be preferable. In addition, since there is little indication for using inotropes in a non-stressed, normovolemic patient, a hypovolemic phase should add to the model to make it more clinically applicable. Therefore this study employs a chronic surgical model to study the effects of epinephrine in both normovolemic and hypovolemic piglets.

Both NEC and acute renal failure (ARF) are significant problems in this patient population. Therefore mesenteric and renal regional blood flows in response to epinephrine and to hypovolemia have been studied.

The search for the ideal inotrope to be used in the neonate continues. The aim of this research is to evaluate the effects of epinephrine on mesenteric and renal blood flow and on blood pressure. Hopefully this will determine the safety and efficacy of epinephrine in the neonate.

Chapter 2

Epinephrine and Adrenoceptors

Epinephrine, one of the principal catecholamines, is released from the adrenal medulla in times of stress and acts on sympathetic effector cells with alpha or beta-adrenoceptors. These receptors are subdivided in to alpha $(\alpha)_1$, α_2, and beta $(\beta)_1$ or β_2-adrenoceptors. Epinephrine has a high affinity for all of these receptors. The effect of epinephrine on a particular effector cell depends on the response characteristics of the effector cell and the predominant type of adrenoceptor found on the cell.

The cardiovascular effects of epinephrine include dose related increases in systolic blood pressure, heart rate, cardiac output (CO), and systemic vascular resistance (SVR). The dose dependent response of the vasculature to epinephrine can be explained by a differing threshold for activation of β_2-receptors than for α-receptors. Epinephrine in low doses (less than 0.2 μg/kg/min) produces β_1 cardiac effects, including increased heart rate and contractility, and β_2 peripheral vascular effects, including vasodilation, resulting in a decrease in SVR. Doses in excess of 0.2 μg/kg/min are associated with increased α-adrenergic effects, which cause peripheral vasoconstriction and an increase in SVR. In therapeutic doses epinephrine stimulates the central nervous system causing apprehension, tremor, restlessness and tachypnea. Since epinephrine does not easily cross the blood-brain

barrier these effects are likely secondary to peripheral actions altering neural feedback (114,137).

Direct effects of catecholamines, such as epinephrine, therefore lead to either vasoconstriction or vasodilation in the subcutaneous, mucosal, splanchnic, renal and skeletal muscle vascular beds, depending on whether alpha- or beta-adrenergic receptor stimulation predominates (83).

Catecholamine therapy based on studies in adults is not necessarily applicable to the infant because of significant differences in cardiovascular and adrenergic physiology (31-37,64,65,84). The stroke volume (SV) in the newborn has a limited capacity to increase because of diminished ventricular diastolic compliance. Consequently, ventricular end diastolic volume can not increase without significant elevation of filling pressure. Since cardiac output equals SV multiplied by heart rate, and stroke volume is relatively fixed, CO in infants is dependent on heart rate. With advancing age and increased ventricular compliance, HR falls while SV increases, leading to an increase in CO (137). Hannon states that the majority of hemodynamic data collected has been from animals subjected to anesthesia or other forms of restraint (69). However, in general, newborn piglets have a higher heart rate and cardiac index (CO divided by weight) than older pigs while stroke index (SV divided by weight) is not age dependent. MAP, SVR, pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR) are lower in newborns despite an initial elevation in PAP and PVR during the first two days of life (69).

Age-related variations in the effect of catecholamines on BP probably result from developmental changes in peripheral α- and β-receptor density. The newborn circulation appears to be under tonic vasoconstrictor tone which decreases with age with increasing beta-receptor density (31-37,57,64,65).

Developmental data on adrenergic-receptor density and the clinical implications of animal studies are limited by differences in adrenergic-receptor ontogeny among different species. The sympathetic nervous system is immature at birth in many species (64,65,137). The newborn responds to changes in BP or hypoxia by integrated baroreceptor and chemoreceptor reflex responses (137). The ontogeny of these responses is known in some animals but there is little information in humans (31-37,64,65). Animal studies in piglets and lambs have demonstrated a diminished contractile and vascular response in the neonate to exogenous catecholamines (94). Plasma catecholamine levels are lower in preterm infants than in term infants suggesting a limited catecholamine stress response in premature infants (82). These studies suggest that neonates may exhibit less endogenous catecholamine response to stress and are also less responsive to exogenous catecholamines than are adults. This differential response may have important clinical effects in the critically ill neonate.

The effects of epinephrine infusion on cardiac output, myocardial oxygen consumption and systemic, coronary and pulmonary vascular resistances in 5-10 day old anesthetized piglets were studied by Barrington (13). Cardiac output increased with doses of 0.2 to 1.6 μg/kg/min and decreased at 3.2 μg/kg/min. Myocardial

oxygen consumption was increased by epinephrine but to a lesser extent than myocardial oxygen delivery. Systemic and pulmonary arterial blood pressures increased at high doses (1.6 and 3.2 μg/kg/min). Systemic and pulmonary vascular resistances decreased at 0.2, 0.4 and 0.8 μg/kg/min and then increased at 1.6 and 3.2 μg/kg/min with a greater increase in the systemic resistance. Barrington concluded that low dose epinephrine acts as a systemic and pulmonary vasodilator in the newborn piglet. Morever he suggests that epinephrine in doses of 0.8 to 3.2 μg/kg/min may be the ideal inotrope for treatment of persistent pulmonary hypertension of the newborn because of its specific pulmonary vasodilatory effect and positive inotropic effect.

Epinephrine may lead to renal and splanchnic vasoconstriction or vasodilation, depending on whether the alpha- or beta-receptor effects predominate. However, even if the predominant response is vasoconstriction, epinephrine-induced improvements in blood pressure may increase renal and splanchnic flow (12).

Bersten studied the dose response effects of a four hour epinephrine infusion in conscious, chronically instrumented adult sheep. Epinephrine resulted in a dose dependent increase in mean arterial pressure and in CO. SVR initially decreased below baseline but then gradually increased. Low to moderate dose epinephrine (5-10 μg/min) did not increase renal vascular resistance (RVR) while high dose (20-40 μg/min) increased RVR markedly initially with a subsequent fall. This fall in RVR with time was accompanied by an increase in renal blood flow (RBF) back to or

above baseline at all infusion rates of epinephrine. Bersten concluded that epinephrine caused RBF to decrease transiently but then return to baseline within 30-60 minutes (23). This return of RBF towards baseline could have significant clinical ramifications for the patient requiring prolonged infusion of inotropes. Whether or not this response is present in the neonate has yet to be determined.

Buckley et al studied the renal circulatory effects of adrenergic stimuli in anesthetized piglets and mature swine including the response to renal nerve stimulation and to exogenous norepinephrine and isoproterenol in sixty-two piglets (35). Transection of the renal nerve resulted in decreased renal vascular resistance in all animals. Renal nerve stimulation revealed age-dependent differences in the threshold and also in the magnitude of increase in renal resistance. There were no significant changes in RBF and RVR during low-frequency stimulation in animals less than 4 days of age. Norepinephrine injection caused age-dependent increases in renal resistance. The increase in RVR was consistently smaller in newborns at each dose tested. In contrast the effect of norepinephrine on blood pressure was equivalent at all ages. Even when the blood pressure increase was large the renal circulation was not a major site of resistance change in piglets less than 2 days. Therefore the renal circulation at birth is less sensitive to renal nerve stimulation and norepinephrine than it is in older pigs. Isoproterenol injection did not consistently alter renal resistance in piglets younger than 1 week despite being a potent vasodilator of the renal circulation in adult swine. Phentolamine attenuated the RVR increase to norepinephrine and to renal nerve stimulation in all animals.

Propranolol attenuated RVR decrease to isoproterenol in older pigs. Buckley concluded that the renal circulation of newborn swine is under active α-adrenergic vasoconstrictor tone with an absence of β-adrenergic vasodilation.

Barrington studied the effects of epinephrine on renal vascular resistance in newborn piglets in an acute model (12). He found that renal vascular resistance fell as the epinephrine dose was increased from 0.2 to 0.8 μg/kg/min and then rose at doses of 1.6 and 3.2μg/kg/min. Renal blood flow however remained significantly above baseline at doses of 0.4 to 3.2. μg/kg/min. He concluded that epinephrine is a renal vasodilator at low doses and causes renal vasoconstriction at high doses. However, because renal blood flow remained above baseline at all doses tested epinephrine infusion should not cause renal ischemia.

Felder et al investigated the maturation of renal α-adrenoceptors in dogs (57). The receptors were identified by radioligand binding using the alpha-1-adrenergic antagonist [^{3}H]-WB-4101. In puppies less than 1 week old, the binding affinity was greater than 3-5 week old puppies but receptor number was similar. They concluded that during maturation there is a decrease in alpha-adrenoceptor density and affinity in the outer cortex of the kidney.

Braatvedt noted that mesenteric blood flow increased during hypoglycemia and that this increase had a close temporal association with a rise in epinephrine levels (30). In a subsequent study he examined SMABF by Doppler ultrasound in adult, male, humans during a 30 minute epinephrine infusion of 10 or 40 ng/kg/min with and without propranolol. SMABF rose in a dose-dependent manner during the

epinephrine infusion alone, but not during the infusion of epinephrine and propranolol. SMAVR fell during the epinephrine infusion alone but rose when combined with propranolol (29). Braatvedt concluded that mesenteric vasodilation is mediated via a beta-adrenergic mechanism during low dose epinephrine infusion.

Epinephrine exerts a number of important metabolic effects through β-receptor activation resulting in increased levels of oxygen consumption, blood glucose, lactic acid and free fatty acids. Hyperglycemia is produced secondary to hepatic glycogenolysis, elevated lactic acid is secondary to skeletal muscle glycogenolysis and impaired hepatic lactate uptake, while elevated free fatty acids is due to lipolysis (83,137).

Catecholamines influence the secretion of renin, insulin, glucagon, calcitonin, parathormone, thyroxine, gastrin, and others. Sympathetic stimulation increases renin release by a direct beta-receptor effect independent of vascular changes within the kidney (83). The subsequent production of angiotensin and aldosterone augments the direct vasoconstrictor effects of the catecholamines and the sodium reabsorption seen with sympathetic stimulation.

The assessment of sympathoadrenal activity involves measuring plasma and urine catecholamine levels and urinary catecholamine metabolite levels. Catecholamines in human plasma may be measured by radioenzymatic isotope derivative techniques or by high performance liquid chromatography in conjunction with electrochemical detection (83). The clinical usefulness of plasma

catecholamine levels is limited by the many factors that alter sympathoadrenal activity, such as change in posture, hypoglycemia or stress.

Basal plasma epinephrine levels are approximately 25 to 50 pg/ml. Plasma levels of 150-200 pg/ml occur with moderate exercise, during and after surgery and with hypoglycemia. Levels greater than 400 pg/ml occur with heavy exercise, severe stress and extreme hypoglycemia (98). The normal resting rate of secretion of epinephrine by the adrenal medulla is about 0.2 μg/kg/min (68).

Blood levels of epinephrine in conscious pigs have been measured with inconsistent results likely related to sensitivity differences in assay procedures between different labs. For example, catecholamine levels measured fluorometrically are higher than those measured by high performance liquid chromatography with electrochemical detection (69). Differences in levels may also be attributable to different experimental conditions. In fact, resting epinephrine levels varied from 80 to 1050 pg/ml in similar sized (approximately 25 kg) pigs in different studies (69). Hanon describes the effect of spontaneous movement in the conscious pig associated with only minor increases in heart rate (HR), mean arterial pressure (MAP) and hemoglobin concentration but a 2.5 fold increase in plasma epinephrine concentration accompanied by marked increases in plasma renin activity and plasma concentrations of ACTH, aldosterone, and cortisol. These effects extended after the spontaneous activity had stopped.

Wade et al studied the effects of physical restraint on conscious 20-25 kg pigs. Animals placed in a sling showed an increase in plasma epinephrine from 69

pg/ml to 337 pg/ml (131). Johansson et al studied the effects of restraint stress on epinephrine levels determined fluorimetrically in adult pigs. Resting epinephrine levels were less than 500 pg/ml but were elevated significantly with physical restraint to levels as high as 6000 pg/ml (74).

These studies show the value of consistent handling of animals and may explain some of the between animal, and between lab, variation in epinephrine levels that is seen in the literature. In addition, the between lab variability in sensitivity of catecholamine assays needs to be identified and controlled for.

Adrenergic-receptors are cell-membrane associated glycoproteins with high specificity and binding affinity for specific catecholamines. $Beta_1$-receptor stimulation affects mainly cardiac function while β_2-receptor stimulation acts on vasculature (vasodilation) and bronchial smooth muscle (relaxation). $Beta_1$-receptors mediate inotropic, chronotropic and dromotropic (increased conduction velocity) activity. Non-selective beta-receptor agonists, such as isoproterenol, stimulate both $beta_1$- and $beta_2$-receptors. Beta-receptor antagonists, such as propranolol, block, or competitively inhibit, the action of agonists (83,137).

Activation of adrenergic-receptors, by catecholamine binding, initiates a cascade of intracellular events that culminate in a measurable response. Activation of beta-adrenergic adrenoceptors involves the activation of adenylate cyclase, an enzyme in the post-junctional cell membrane associated with the β-receptor which catalyzes the conversion of adenosine triphosphate to cyclic adenosine monophosphate (cAMP). This conversion to cAMP is regulated by another plasma

membrane bound guanine nucleotide protein (G protein). Increased levels of cyclic AMP (a second messenger) in the effector cell activate a protein kinase that catalyzes the conversion of a previously inactive enzyme to an active enzyme by the process of phosphorylation. This sequence of events initiates a response (114,122).

The alpha-receptor mediates vasoconstriction, intestinal relaxation, and pupillary dilation. $Alpha_1$-receptor stimulation leads to blood vessel vasoconstriction. Phenylephrine and methoxamine are selective $alpha_1$-receptor agonists, while prazosin is a selective $alpha_1$-receptor antagonist. The second messenger for $alpha_1$-mediated processes has not been identified with certainty but may involve changes in intracellular calcium concentration or in membrane phosphatidylinositol turnover (83). $Alpha_2$-receptor stimulation effects are less well defined but include presynaptic feedback inhibition of norepinephrine release and inhibition of insulin secretion. Specific $alpha_2$-receptor agonists include clonidine and alpha-methylnorepinephrine. Yohimbine is a selective $alpha_2$-receptor antagonist. The second messenger for the $alpha_2$-receptor system involves inhibition of adenylate cyclase (83).

Changes in adrenergic receptors occur under a number of different physiologic conditions. These changes can be assessed by studies of peripheral tissue sensitivity or by radiolabeled adrenergic-receptor agonists and antagonists. Prolonged exposure to alpha- or beta-adrenergic agonists decreases the number of corresponding adrenergic-receptors on effector cells (71,78,83). While the exact mechanism of this regulation remains to be defined, internalization of the beta-

receptor within the cell (internal translocation) occurs during agonist exposure in some systems leading to a decrease in receptor number (28,71,83). Changes in agonist concentration may also affect receptor binding affinity for the agonist. Adrenergic-receptors that utilize adenylate cyclase for the second messenger (beta and $alpha_2$) exist in low and high affinity states with agonist exposure leading to a decrease in the number of receptors in the high affinity state. These decreases in receptor number and affinity likely contribute to the diminished physiologic response that occurs after prolonged exposure of an effector tissue to adrenergic agonist, a phenomenon referred to as tachyphylaxis, tolerance, refractoriness or desensitization (28,71,78,83,362).

Homologous regulation is the change in adrenergic-receptors induced by adrenergic agonists. For example, administration of beta-agonists induces specific desensitization of heart muscle to readministration of the same agonists but not to drugs that do not act via beta-receptors. Receptor specific desensitization involves at least two separate mechanisms. The first, which develops rapidly (within minutes) and is rapidly reversed upon removal of the hormone, functionally uncouples receptors from the G protein and thereby reduces their ability to stimulate adenylate cyclase. The second process involves actual reduction in the number of receptors on the cell membrane, a process termed receptor down-regulation. This down-regulation process may require hours to occur and is not readily reversible (28,71,83).

Heterologous regulation is the change in adrenergic-receptors in response to factors other than adrenergic-agonists, such as enhanced alpha-receptor affinity in response to low environmental temperature. The sensitivity of one hormone is regulated by a second hormone acting through a different set of receptors, such as the increased responsiveness of many tissues to epinephrine that is produced by glucocorticoids (83).

The beta adrenergic-receptor linked adenylate cyclase system has received much study. It is known to undergo agonist-induced desensitization during exposure of intact cells to catecholamines and other hormones (71). Elevation of cAMP levels in intact cells by agonists is transient with nucleotide levels usually returning to near basal levels within 30-60 minutes despite continued presence of hormone. Rechallenge of the beta-receptor by an agonist results in a reduced responsiveness of the cAMP generating system. Although this change may come as the result of alterations at any one of several steps, the most important modification appears to be a reduction in the rate of cAMP synthesis. The beta-adrenergic receptor-linked adenylate cyclase seems to comprised of at least two proteins; a cell surface receptor and a guanine nucleotide binding protein (G protein). The G protein serves a regulatory function in coupling agonist-receptor interaction to activation of the enzyme. Adrenergic-agonists bind to beta-receptors in a high affinity complex made up of hormone, receptor and G protein which acts as an obligatory intermediate through which hormones stimulate the rate of activation of adenylate cyclase by guanine nucleotides. Catecholamine-induced uncoupling of

the adenylate cyclase system during desensitization also results in a decrease in the extent of high affinity binding (71).

Catecholamine-specific (homologous) desensitization appears to proceed by a common mechanism in a variety of systems. There is evidence that rapid desensitization can occur within minutes that leads to uncoupling of the beta-receptor/adenylate cyclase system, this change occurs without a change in receptor number. One mechanism for desensitization may be the internalization of the hormone-receptor complexes within the cell such that subsequent released hormones would be unable to interact with the receptor. This internalization is likely a reversible process and may be related to the low affinity population of receptors that have been detected (71).

Catecholamine-induced heterologous desensitization of the adenylate cyclase system is a well documented phenomenon that occurs in a variety of cell types. In contrast to homologous desensitization changes in receptor number do not appear to be involved (71).

Long-term exposure to catecholamines results in a measurable loss of beta-adrenergic receptors. In several tissues this process has occurred over a period of hours rather than the almost instantaneous occurrence of decreased adenylate cyclase activity. The likely sequence of events therefore would be as follows: activation of receptors, uncoupling of receptors, change in membrane form of receptors (internalization), and finally loss of the receptor (71)

In vivo studies are lacking to date. In most studies catecholamine desensitization has occurred after increased receptor stimulation for hours or days while in vitro studies have shown that a more rapid response may be present (71). Long-term treatment of asthma with beta-agonists has resulted in desensitization of the adenylate cyclase system in lymphocytes of these patients (66,99). Inotropic and chronotropic sensitivty of the heart to catecholamines is greatly reduced within 2 hours of infusion however most studies of the adenylate cyclase system have looked at longer infusion times (40,71).

It is likely that beta-adrenergic receptors exist in such a fashion that maximal occupancy of the receptors is not necessary to elicit a maximal biologic response. If desensitization has occurred the efficacy of agonists for elevation of intracellular cAMP will be reduced. Therefore, more receptors will need to be occupied to produce the same elevation in cAMP leading to the necessity of a higher concentration of hormone to elicit the same biologic response (71). Unfortunately there is little information on the beta-receptor response in the peripheral vasculature which may be vital to understanding the effects of desensitization on regional blood flow.

Desensitization may therefore be a factor in the critically ill newborn who is requiring increased doses of inotropes rather than a worsening of the patients hemodynamic status.

While the beta-receptor adenylate cyclase system has received a great deal of study, there is relatively little known about the mechanism of action of the alpha-

receptors. The second messenger has yet to be isolated although there is some evidence that calcium may play a role (83). There is little known on the extent to which alpha$_1$-adrenergic peripheral vascular desensitization occurs and the mechanisms involved. Carrier et al studied the desensitization of rabbit aorta in an in vitro experiment. They concluded that desensitization to norepinephrine occurred following 10 minutes of incubation with norepinephrine and that this desensitization was specific for the alpha-receptors (38).

Lurie et al studied alpha$_1$-receptor mediated desensitization using in vitro rabbit aortas. They detected desensitization after a 7 hour incubation with epinephrine which was specific for alpha-receptors. Furthermore, using a radiolabeled alpha$_1$-receptor antagonist they found that desensitization was not mediated by a decrease in receptor number nor affinity but was associated with a decreased stimulation of phosphatidylinositol turnover. They concluded that desensitization of alpha$_1$-receptor mediated vasocontriction was not associated with changes in receptor number or affinity but with changes in receptor coupling (89). This decreased receptor sensitivity would likely mean that more receptors need to be occupied in desensitized smooth muscle to generate the same response seen in a control vessel.

Kiuchi studied whether chronic alpha$_1$-adrenoceptor stimulation would induce desensitization of peripheral vascular responses to acute alpha$_1$-receptor challenges by norepinephrine and the selective alpha$_1$-agonist amidephrine mesylate in the conscious dog. Desensitization was observed after one week of chronic

alpha$_1$-receptor stimulation, furthermore, alpha$_1$-receptor density and affinity as determined by radiolabeled prazosin binding was decreased. Kiuchi concluded that not only the reduction of receptors but also other agonist-induced alterations in coupling to second messengers may contribute to the mechanism of desensitization to alpha$_1$-adrenergic receptor-mediated pressor and vasoconstrictor responses (78).

Maze et al studied desensitization in awake rabbits (2.5-3.0 kg) after a 2 hour epinephrine infusion. Epinephrine was infused at 1 μg/min which resulted in a 15-fold increase in plasma epinephrine levels as determined by the high performance liquid chromatography technique. They determined that desensitization to subsequent alpha$_1$-receptor stimulation occurred within 2 hours of the onset of epinephrine infusion. In addition, this effect was only slowly reversible after removal of the agonist (97).

Hiremath et al examined the role of the endothelium in desensitization using the aortas from pheochromocytoma-bearing rats and aortic segments exposed to phenylephrine for 6 hours. They observed a decreased desensitization response in vessels lacking endothelium and postulated that this effect may be secondary to the release of endothelial derived relaxing factor. In addition, they demonstrated that this endothelium dependent response was not due to prostaglandins or adenosine as indicated by the inability of indomethacin and 8-(sulfophenyl)theophylline to modify contraction in the desensitized vessels (73).

Up-regulation of adrenergic receptors is the process whereby there is an increased receptor density or hormone binding affinity. This may be in response to a

decreased circulating hormone concentration or to other factors such as steroids which may give rise to beta-receptor up-regulation (28). If a sympathetic nerve is destroyed, the innervated organ becomes more and more sensitive to norepinephrine: this is referred to as denervation sensitivity and is at least partially due to an increase in the number of receptors in the postsynaptic membranes of the effector cell (68).

Overall there are are few studies on the use of epinephrine in the neonate. Epinephrine is associated with an increase in CO, MAP and SVR, however these effects are dose related. While high dose epinephrine appears to cause mesenteric and renal vasoconstriction, low dose epinephrine may have beneficial effects on regional blood flow via beta-receptor stimulation. In addition, adrenergic-receptor regulation in peripheral vasculature, secondary to exogenous catecholamines, may give rise to significant effects which remain to be delineated. Further laboratory and clinical investigation is required to establish the safety and efficacy of epinephrine use in the neonate.

Chapter 3

The Critically Ill Neonate

Potential complicating factors in the treatment of a critically ill neonate include problems such as necrotizing enterocolitis (NEC), acute renal failure (ARF) and persistent pulmonary hypertension (PPH). While epinephrine is known to increase CO and MAP, many physicians remain reluctant to use it because of the possible risk of mesenteric or renal ischemia which could possibly lead to, or worsen, NEC or ARF. Whether or not epinephrine actually puts the neonate at risk for these complications is an area that requires further study.

NEC is usually seen during the first two weeks of life in premature infants born at less than 36 weeks gestation (79). There are several potential theories of the pathogenesis of the disease (9,46,79,81,104,127). NEC typically occurs after a period of asphyxia which is then followed by resuscitation, a latent period and subsequent gastrointestinal (GI) findings. NEC is usually preceded by severe postnatal cardiorespiratory distress with hypoxemia, hypercarbia, and acidosis. Symptoms include gastric retention, bilious vomiting, abdominal distension and bloody diarrhea. Hemorrhagic and necrotic lesions, with or without perforation, are commonly found in stomach, ileum and colon. The common pathologic findings in NEC include coagulation (ischemic) necrosis, inflammation and bacterial overgrowth (9).

One of the common proposed theories is the ischemic insult theory which basically states that a clinical condition leading to low mesenteric blood flow leads to the above changes. Epinephrine has the potential to decrease mesenteric blood flow which may worsen a pre-existing low-flow state, however epinephrine also has the potential for beta-receptor mediated vasodilation. The actual effects of epinephrine on mesenteric blood flow in the critically ill neonate have not been clearly delineated.

In a model of NEC, Touloukian induced postnatal respiratory distress in piglets and subsequently measured intramural and mucosal gut perfusion (127). He noted that the entire alimentary tract, except the esophagus, responded to asphyxiation by a decrease in perfusion, particularly a decrease in mucosal perfusion. Touloukian concluded that asphyxia and resuscitation are accompanied by reduction and then rebound above normal blood flow. Focal hemorrhage within the mucosa results from a combination of increased capillary fragility produced by gut ischemia and vascular congestion of the gut in the resuscitated patient. Focal mucosal necrosis, invasive bacterial proliferation and transmural inflammation result in GI dysfunction and subsequent entercolitis. The ill neonate may respond to supportive medical therapy or progress to intestinal necrosis and perforation.

Crissinger reviewed the regulation of blood flow and oxygenation in the immature intestine (46,47). Many changes in blood flow regulation in piglets occur in the first 24 hours of life. Autoregulation is the ability to maintain flow constant as perfusion pressure changes. Piglets less than 2 weeks of age appear to be unable to autoregulate intestinal blood flow, but can after one month (32,37,72,105). Despite

this lack of autoregulation, one day old piglets are capable of increasing intestinal oxygen extraction (105) so that oxygen uptake increases to a similar extent post-prandially when compared to older pigs (47). Despite this ability to increase oxygen extraction, without the maintenance of blood flow, the immature intestine may be at increased risk for ischemia.

While autoregulation of the mesenteric blood flow appears underdeveloped in the newborn piglet, reactive hyperemia seems to be present from birth (32,37). Reactive hyperemia is the elevated blood flow response seen after a vessel has been occluded for some time. This reactive hyperemia, while restoring the metabolic deficits of the tissue, may lead to reperfusion injury and has been implicated as one of the factors in NEC (79).

As in the renal circulation, the intestinal circulation at birth appears to be under tonic sympathetic vasoconstrictor tone in piglets (33). The delayed onset of autoregulatory escape (the ability to increase blood flow despite continued vasoconstictor stimulation) from postganglionic mesenteric nerve stimulation may be due to a postnatal delay in beta-receptor mediated vasodilation capability similar to the renal circulation.

Crissinger studied SMA collateral blood flow and found it also to be age dependent (48). Occlusion of a distal branch of the SMA in one day, compared to one month, old piglets resulted in a greater decrease in blood flow. This lack of collateral flow may be a risk factor for NEC as well.

The pathophysiology of acute renal failure in the neonate can be divided into pre-renal, intrinsic, and post-renal causes, as in the adult (2). Pre-renal acute renal failure can occur with hypovolemia or hypotension. The potential risk of further diminished RBF and consequent renal ischemia has been a major fear with epinephrine use in the hypotensive neonate. Although dopamine is widely used in hypotensive adults, partly because of its selective renal vasodilation effects, there is evidence that this effect is not seen in neonates (107,108).

Renal autoregulation has not been observed in piglets until one week of age. (37). The inability to maintain renal blood flow during hypotension at birth may put the newborn infant at risk for ARF, particularly the premature infant. In addition the newborn piglet appears to be under tonic vasoconstrictor tone (35,57). The combination of the above factors puts the critically ill neonate at risk for ARF. The dose-related and temporal response to epinephrine infusion in this setting requires further differentiation.

With persistent pulmonary hypertension of the newborn, pulmonary vascular resistance is high while cardiac output is low. A drug that has selective pulmonary vasodilation or selective systemic pressor and positive inotropic properties would be ideal (13). Barrington studied the circulatory effects of epinephrine and dopamine in newborn piglets (14). Epinephrine had a more advantageous effect on the PVR to SVR ratio when compared to dopamine. The combination of increased CO and MAP as well as a propensity to increase SVR more than PVR may make epinephrine the best available choice for PPH in the neonate.

The problems of NEC and PPH are unique to the newborn. In these settings, it may not be appropriate to base the use of inotropes on studies which were done in adults. While acute renal failure is seen in both newborns and adults, there is a growing amount of evidence to suggest that the newborn and adult renal vasculature may respond differently to exogenous catecholamines. Further animal models and clinical trials need to be performed to develop a better understanding of the effects of inotropes in the critically ill neonate.

Chapter 4

The Use of a Chronic Model

Several studies have examined the effects of catecholamines in young animals. A potential confounding variable in many studies is the effects of anesthesia. An acute model is performed while the animal is under anesthesia, as opposed to a chronic model where the animal is not anesthetized. In addition, studies of anesthesia effects have generally been done in adult models and may not be applicable in newborns because of differences in response to stress and other homeostatic mechanisms.

There are a number of effects associated with anesthetic administration. Eisele reviewed the use of inhalant anesthetics in swine research (55). She found that halothane produced a decrease in cardiac output and mean arterial pressure in swine in a dose dependent manner. Piglets under one week of age have been shown to be sensitive to its hypotensive effects (45). Halothane sensitizes the myocardium to catecholamine induced arrhythmias and can trigger malignant hyperthermia (55).

Halothane is also known to produce a marked decrease in splanchnic blood flow secondary to a decreased CO and subsequent lower perfusion pressure (15,88). Bailie reviewed the effects of anesthesia on renal and cardiovascular function in the newborn piglet (5). All of the anesthetics tested (including halothane, pentobarbital and nitrous oxide) caused a decrease in cardiac output and an increase in total

peripheral resistance. Halothane caused a decrease in heart rate and systemic blood pressure, variable changes in renal blood flow and an increase in plasma renin levels. Pentobarbital caused an increase in systemic blood pressure.

Manohar studied the hemodynamic effects of anesthesia in swine using the microsphere technique (95). Nine healthy 20-23 week old pigs were studied. With halothane-oxygen anesthesia, a dose dependent depression of MAP, SV and CO was observed in all pigs. SVR was unchanged from baseline. The effects on renal flow were variable, however there was a definite decrease in flow to the small intestine. Clinically useful concentrations of halothane caused hemodynamic alterations in almost all tissues. Most of these changes were similar to those caused by other modern inhalation anesthetics such as isoflurane, enflurane and sevoflurane.

In addition to the above, anesthetic agents may alter the responsiveness to catecholamines. Berne showed that dogs anesthetized with pentobarbital or chloralose were more sensitive to epinephrine-induced renal vasoconstriction than the conscious dog (20).

Zaritsky points out that studies in anesthetized animals may not be clinically applicable because of anesthetic-induced suppression of the endogenous catecholamine response and homeostatic hemodynamic reflexes (137).

In summary, the use of anesthesia may affect the cardiovascular response to exogenous catecholamines in an unpredictable fashion. The use of a chronic model is not complicated by this potentially confounding variable and may therefore be preferable to an acute model.

Chapter 5

The use of a porcine model

The newborn pig has been used as a biomedical research model for studies in embryology, teratology, nutrition, metabolism, immunology, physiology, biochemistry, endocrinology and hematology. Previous studies have also demonstrated similarities in control of blood pressure, heart rate, regional blood flow, response to stress and gastrointestinal physiology. Compared with other newborn models such as the dog, cat or lamb, the newborn pig appears to be the most similar, overall, to the human neonate.

Although there are pig and human gestational differences (gestation period and number of young), the development of the fetal pig is similar to that of the human fetus and has become the standard for mammalian embryologic study (39). Pigs have a degree of maturity at birth similar to human infants, their hematologic values are similar and their growth patterns are comparable (123).

The newborn pig has no circulating immunoglobulins and must acquire them from the sow's colostrum. The colostrum-deprived piglet remains hypogammaglobulinemic for several weeks, providing a good model for studying the developing immune system (120).

Newborn pigs and human neonates both show a drop in body temperature at birth followed by a rise; both shiver and have little thermal insulation and the

metabolic rates of both increase in the first few days following birth. However, the piglet has a lower birth weight, more rapid growth rate, higher body temperature and metabolic rate, decreased ability to perspire and a more limited fat reserve (100).

There has been extensive study of nutrition using piglets as the animal model. Newborn pigs have been used to evaluate infant formulas and milk substitutes as well as models for malnutrition (110). Being a true omnivore, the gastrointestinal physiology in the pig is felt to be very similar to humans (123).

Swindle reviewed the use of swine in surgical research. Porcine models have been used extensively in transplantation studies of the heart, pancreas, kidney, liver and intestine. Both dogs and pigs are used for heart transplantation, however the growth of the heart is more similar to humans in the pig than the dog. Swine have been used to study rejection phenomena in kidney transplants. Liver and small bowel transplantation studies in swine are useful because of the physiologic similarity with humans, including rejection phenomena (123).

Swine and humans respond to exercise in a similar manner (118,119); there is a decrease in blood flow to the major visceral organs, including the kidney, however this does not occur in dogs.

Porcine cardiovascular models are used because the anatomy and physiology is similar to man, particularly in regards to the coronary arteries and reaction to ischemia. In addition, the pig has similar vascular anatomy to the human as well as a similar blood clotting system (123).

The kidney and urinary tract of swine is more similar anatomically and physiologically to man than other nonprimate animal models (123). The development of the kidney in the newborn pig has been extensively studied. The maturation of renal function in piglets and human infants is comparable (103). Kidneys of newborn pigs and humans are characterized by both low glomerular filtration rate and low renal blood flow when compared to mature pigs and humans. The anatomical development of the kidney is also similar. At birth, the outermost portion of the renal cortex is not developed. During the first month of life, new glomeruli are formed in the outer cortex. Glomerular filtration rate is 75 ml/min/m^2 in humans and 72 ml/min/m^2 in pigs. The maturational increase in proximal convuluted tubule size is 10-15 fold in man and 10 fold in pigs.

Terris reviewed the use of swine as a model of renal physiology and nephrology (125). Swine are the only mammal with the exception of the dwarf water buffalo, with kidneys morphologically similar to humans. The kidneys are multipyramidal with undivided cortex but several medullary structures. Each medullary pyramid forms a separate papilla or crest. Usually there is fusion resulting in a compound papillae. This similarity in structure is advantageous in studies of vesicoureteral reflux, pyelotubular and pyelolymphatic backflow and pyeloureteral dynamics. Other similarities include percentage of long looped nephrons (pig 3%, man 14%), maximal urine concentration (pig 1080 mOsm/L, man 1160 mOsmol/L), maximal urine/plasma osmolal ratio (pig 3.7, man 4.0), and acetylation of PAH (102).

As mentioned the pig is ideal for studying vesicoureteral reflux because of the morphologic similarity to man. Renal lymphatics, in the pig, drain the renal interstitium and are important in the setting of acute ureteral obstruction where the sudden elevation in pressure can lead to calyceal fornices rupture with extravasation into the interstitium (pyelolymphatic backflow). These lymphatics drain into perihilar lymph nodes and then to the cisterna chyli and are similar to man (43).

An increase in RBF is a maturational process common to mammals. Maturational characteristics of fetal and neonatal kidney are similar to the newborn infant. In piglets 6 hours to 45 days of age, Gruskin showed that mean RBF increased from 43 ml/min/m^2 to 760 ml/min/m^2 (67). This large increase was due to an increase in CO (900 to 6500 ml/min/m^2) as well as a decrease in RVR. By six weeks of age, CO was at adult levels and any further increases in RBF were secondary to decreased RVR. This is in agreement with Buckley's conclusion that newborn piglets are under tonic renovascular vasoconstrictor tone (35).

The porcine model is extensively used in laboratory research. Swine have been described as the best physiologic model among laboratory species for comparison to the human infant (123). This is particularly true in regards to the renal and vascular systems which are under close scrutiny in the study of the effects of epinephrine in the neonate.

Chapter 6

The hypovolemic model

Several studies have documented the effects of catecholamines in normovolemic lab animals. Such effects may be of questionable relevance in the clinical setting since the non-stressed subject may respond much differently than the stressed, hypovolemic or shocked subject.

Weiskopf looked at the cardiovascular, endocrine and metabolic responses of conscious swine to hemorrhage (133). The pigs (approximately 20 kg in weight) were bled 30% of their blood volume and observed for 30 minutes. HR increased while CO and stroke volume decreased. Plasma renin activity and plasma concentrations of epinephrine, norepinephrine, and vasopressin increased. Plasma epinephrine increased from 227 pg/ml in normovolemic pigs to 683 pg/ml in hypovolemic animals. SVR and PVR increased markedly. MAP and PAP decreased; body temperature, oxygen consumption and plasma lactate increased.

Bailey et al studied the effects of cardiogenic shock in 10-20 kg piglets in an acute model (7). By inducing graded degrees of cardiac tamponade they showed a fall in MAP with decreasing cardiac output and a corresponding rise in total peripheral resistance. They also studied a hemorrhagic model; after 15% hemorrhage, arterial pressure rose from 95 to 97 mmHg, while SVR rose from 3.8 to 4.5 mmHg/ml/min x 100g. In association with these systemic changes, SMA blood flow decreased 2%, while SMAVR rose 5%. When cardiac tamponade was

superimposed on the 15% blood loss, progressive decreases in cardiac output were accompanied by profound diminution in SMA blood flow which could be ascribed to both decreased MAP and to splanchnic vasoconstriction. Indeed, splanchnic vasospasm was more profound than the noted increase in SVR and was considered a possible factor leading to nonocclusive mesenteric ischemia. In addition, Bailey found that this vasospasm could be abolished by ablation of the renin-angiotensin axis (by using angiotensin converting enzyme inhibitors) but not by sympathetic blockade. Further delineation of the possible benefits of blocking the renin-angiotensin system are required.

Dyess studied the redistribution of organ blood flow after inducing hypovolemia in newborn piglets in an acute model (54). Piglets aged 1-2 days and 7-14 days were used to study the redistribution of organ blood flow after 25% acute blood loss. The animals maintained flow to the heart and CNS but had significantly decreased flow to the kidneys and splanchnic organs. In addition, the vascular resistance was increased to the viscera significantly over baseline. In the GI tract, the small intestine was affected most severely with a decrease in blood flow, particularly to the mucosa. Mucosal ischemia has been implicated as a causative factor in necrotizing enterocolitis and bacterial translocation.

Ramenofsky studied the response to hypovolemia of neonatal piglets (5-7 days old) in an acute model (111). The animals were bled 20% of their estimated blood volume followed by microsphere injection. Cardiac output was decreased, as was flow to the gastrointestinal tract, kidneys, skin and muscle, but flow was

maintained to brain and heart tissue. There was no change in heart rate post-hemorrhage although there was a 30% drop in both systolic and diastolic pressure. Ramenofsky concluded that differential organ perfusion occurred in the hypovolemic neonatal pig and that this represented a vasoconstrictive response that was possibly mediated by the autonomic sympathetic nervous system.

Buckley studied the cardiovascular effects of graded arterial or venous hemorrhage in developing swine between one and sixteen days old in an acute model (31). The pigs were bled (twenty ml/kg) and regional blood flow was measured with electromagnetic flow probes. Tachycardia occurred in most animals. Decreases in aortic pressure to arterial, but not venous, hemorrhage were age dependent. Buckley concluded that there was a progressive maturation-related compensation to the stress of arterial hemorrhage.

Legal examined the changes in hemodynamics in newly born pigs bled one third of their measured blood volume (84). Decreased heart rate, MAP, and CVP in newborn pigs after hemorrhage were observed, with recovery of heart rate, MAP and CVP and a decrease in hematocrit during the subsequent twenty-four hours most probably due to the replenishment of plasma volume. The pattern of plasma volume restoration was similar to that of the adult human and adult pig except that it occurred faster and with a tendency to overcompensate. Drucker states that the restoration of blood volume following hemorrhage in man or in experimental animals consists of two phases: a rapid first phase with partial restitution which lasts 2-6 hours and a slower second phase which requires 12-48 hours for the completion of

blood volume restoration (53). Legal concluded that the cardiovascular regulatory system at birth is different from the adult but that at least some of the homeostatic processes involved in restoration of blood volume and plasma proteins are functional in the neonate (84).

Rowe studied the hemodynamic responses of the neonatal puppy, aged one to nine days, to acute hemorrhage (115). The animals were bled 35% of their blood volume over ten minutes. Pulse rate, cardiac output, and MAP all decreased while CVP was unchanged and systemic vascular resistance increased.

Gootman studied the cardiovascular response to hemorrhage in piglets aged birth to 2 months in an acute model (65). Regional, including mesenteric and renal, blood flows were recorded with electromagnetic flow transducers and vascular resistances were calculated. The control data showed age-dependent differences in HR, MAP, regional blood flow and vascular resistances. Pigs were bled up to 15 ml/kg and the same variables were measured approximatley 5 minutes later. Hypovolemia resulted in tachycardia and increased renal and mesenteric resistance. The increase in RVR was significantly greater in 2-4 day old than in 1 week old piglets. The increase in mesenteric resistance was similar in both age groups. The differential response in the renal circulation may be related to post-natal maturation of adrenergic vascular responses.

Previous studies describing changes in MAP, CVP and heart rate after blood loss in neonates have yielded variable results. These differences may be due to: variations in the amount and rate of blood withdrawal, variations in the time intervals

measured, age-related differences, species differences, different routes of withdrawal or the effects of anesthesia. In general however, most studies have shown a decrease in MAP and CO as well as reduced mesenteric and renal blood flow. Normovolemic and hypovolemic neonates may have a differential response to exogenous catecholamines, however this subject has received little study to date.

Chapter7

Summary

The ideal inotrope for use in neonates remains to be found. Despite widespread clinical use of inotropes there have been few laboratory or clinical studies on the subject. In particular, alternatives to the traditional first choice inotrope, dopamine, need to be explored because of potential adverse effects (14,52,80,107). Epinephrine is known to have reliable effects of increasing MAP, CO and SVR in a hypotensive patient. Epinephrine's major downfall has been the potential risk of renal and mesenteric ischemia, however the effect of continuous epinephrine infusion has received very little study (23), particularly in the neonate. When considering the neonatal problems of NEC, ARF and persistent pulmonary hypertension, an inotrope that preferentially increases SVR over PVR while increasing CO as well as mesenteric and renal blood flow, would be ideal. Epinephrine deserves study as it has the potential to do all of the above.

Chapter 8

Materials and Methods

Surgical procedure

Sixteen piglets, one to three days old, of mixed western breed of either gender were used . The piglets were anesthetized with 5% halothane and then were allowed to breath spontaneously with a maintenance of 1- 2% halothane. Heart rate and arterial oxygen saturation (Nellcor pulse oximeter, Hayward, California) were monitored continuously. Intra-muscular Pen-Di-Strep (rogar/STB Inc.), a combination of penicillin and streptomycin, 0.3 ml, was given pre-operatively. A left sided neck incision was performed and the left external jugular vein and left common carotid artery were cannulated with double (Arrow, 4F pediatric two-lumen Reading, Pennsylvania) and single (Argyle, 5F umbilical vessel catheter Sherwood Medical, St. Louis, MO) lumen catheters respectively. The lines were heparinized and brought out subcutaneously through an incision just off the midline of the back. The neck incision was then closed with interrupted sutures. A left flank incision was performed at the costal margin posteriorly. The aorta and left kidney were exposed via a retroperitoneal approach. The superior mesenteric artery (SMA) was exposed using minimal dissection and a two or three mm ultrasonic flow probe (Implantable Perivascular flow probes - Transonic Systems Inc. Ithaca, New York) was placed around the artery and then secured to surrounding muscular tissue. The left renal artery was then exposed, again using minimal dissection, and a one or two mm probe

was set in position and secured to surrounding muscular tissue. The ends of the probes were brought out subcutaneously through the back incision. The flank and back incisions were then closed with interrupted sutures. The probes and vascular catheters were grouped together and placed in a "knapsack" (an empty 50 ml normal saline bag) which was sutured to the piglets back. The piglet was wrapped in gauze dressings and allowed to wake. Total operative time was approximately one hour. Oral acetaminophen was used as needed, as determined by activity, appetite and overall appearance, for post-operative analgesia. The piglets were fed as tolerated post-operatively.

Experimental procedure

The piglets were returned to the laboratory for monitoring approximately 48 hours post-instrumentation. During the 48 hr period the piglets were handled often to try to minimize the stress reaction on the day of recording. The conscious, non-sedated piglet was placed in a modified cat box (CDMV Inc. St. Hyacinthe, Quebec) lined with paper scraps into which the piglet would settle and then rest for the duration of the monitoring. There were no other external restraints. Baseline measurements were not taken until a settling period of about 30 minutes had elapsed. All catheters were flushed with heparinized saline. The carotid line was connected to a pressure transducer which was amplified by a HP 78342A clinical monitor (Hewlett Packard) to yield mean arterial pressures. The double lumen venous catheter was connected to a normal saline line at 4ml/kg/hr, an epinephrine line and intermittently to a pressure transducer to give central venous pressure.

The flow probes were connected to a flow meter (T206 dual channel small animal blood flow meter - Transonic Systems Inc. Ithaca, New York). The analog data recorded from the vascular catheters and flow probes was digitized by an analog to digital converter (Datatranslation, model DT2801A Mississauga, Ontario) and recorded using a 486/25 megahertz personal computer running the ASYST computerized software data acquisition program and stored for future analysis.

After recording baseline values, epinephrine infusion was started. Epinephrine was infused using a micro-flo-guard 8500 rotating disk volumetric infusion pump (Travenol Labs Inc. Deerfield, Illinois) while normal saline was

infused with an Imed 965 micro volumetric infusion pump (Imed corporation San Diego, California).

The doses of epinephrine tested were 0.2, 0.4, 0.8, 1.6, and 3.2 μg/kg/min in randomized order. Each new dose of epinephrine was infused over a 20 minute interval, the last 10 minutes of which was used for recording purposes. After each infusion there was a 10 minute wait or lag interval and a repeat baseline was then recorded for 5 minutes before the next dose was administered. Epinephrine is rapidly cleared with a half life of approximately 3 minutes (59).

After all five doses were infused the hypovolemic section of the monitoring was started. Approximately 25% of the piglets blood volume (20 ml/kg) was withdrawn. A new baseline was recorded and the five doses of epinephrine infused in the method described above. At the completion of the monitoring the piglet was euthanized using sodium pentobarbital.

A total of 18 piglets were studied. Two piglets died secondary to small bowel infarction after the SMA flow probe twisted and occluded SMA flow. In the remaining 16 piglets there were 14 mesenteric and 9 renal blood flow recordings. Technical problems led to the lower number of recordings than piglets.

The major technical problem involved probe migration off of the renal artery. In addition, particularly early in the study, probe size was not always ideally matched to vessel size, leading to sporadic, unusable data. In all cases, an autopsy was performed to ensure that there was no evidence of mesenteric or renal ischemia.

In the hypovolemic group there was variation in the baseline values determined after each epinephrine dose (see results and discussion). To try and explain this phenomenon a second group of five piglets was studied yielding five SMA and three renal recordings The same surgical and experimental procedures were used with the following exceptions. Monitoring was begun with a one hour long baseline determination. The piglets were then bled 20 ml/kg and monitoring was continued for another 3.5 hours which was divided into ten minute segments for analysis. No epinephrine was administered.

The perivascular flow probes used were the Transonic type patented by Cornell University. The system uses an ultrasonic transit-time principal to sense liquid volume flow in vessels. These probes have an accuracy of +/- 15%. This transit time blood flow probe gives a direct measurement of the net volume flow through the acoustic window of its implanted sensor. This is in contrast to the doppler flow meter which senses blood velocity and makes determination of vessel diameter critical for determination of volume flow. Electromagnetic flow probes are commonly used however a precise fit to a vessel is required. Accuracy of the Transonic probes is, in principle, independent of flow profile and vessel dimensions, and is insensitive to minor misalignment of the vessel within the sensor (11). The transit time ultrasonic flow probes compare favorably with the microsphere method of measuring blood flow (11,112). While the microsphere technique probably remains the gold standard by which to compare other methods of blood flow there can be problems with uniform mixing, second pass effects and disruption of

hemodynamics. In addition, flow probes have the advantage of yielding continuous blood flow readings.

Calculated values

The SMAVR and RVR were calculated using Ohm's law:

$$SMAVR = (MAP-CVP)/SMABF$$

$$RVR = (MAP - CVP)/RBF$$

Statistical methods

The sample size required was calculated using the following predetermined parameters: type one error rate (alpha error) of 0.05, type two error rate (beta error) of 0.20 and an estimated variability of 25% in data, expressed as standard deviation. This resulted in an estimated sample size of ten to provide adequate statistical power to detect a 35% change in blood flow (12,13,42,92). The data was analyzed using one way repeated measures analysis of variance. The mean percentage change from baseline values at each drug dose was using Sigmastat software (Jandel Scientific, San Rafael, California). The posteriori test used was the Student-Newman-Keuls multiple comparisons procedure to isolate the significant differences in dose response (136). A p-value of less than 0.05 was considered significant.

Chapter 9

Results

Mean arterial pressure (MAP), superior mesenteric artery blood flow (SMABF), superior mesenteric artery vascular resistance (SMAVR), renal blood flow (RBF) and renal vascular resistance (RVR) in relation to epinephrine dose (μg/kg/min) and hypovolemia were examined.

Table1 lists the baseline values of these variables for the sixteen piglets. Baseline values for MAP, SMABF, SMAVR, RBF, and RVR in this study were consistent with other studies in newborn piglets (12-14,31-37,64,65). Blood levels of sodium, potassium and glucose were determined at the time of hemorrhage. These values are also similar to those reported previously for piglets of similar age (128).

Table 2 shows the average baseline during the period of monitoring in the normovolemic piglet as well as the effects of epinephrine on blood pressure and regional blood flow. The initial post-hemorrhage baseline values are included to show the initial response to hemorrhage. Because the baseline was found to change with time after hemorrhage, the average baseline values are quite different from these initial baseline values. The effects of epinephrine in the hypovolemic setting are also included in this table.

Table 3 reveals the percentage change from baseline, for each epinephrine dose, in blood pressure and regional blood flow, in both the normovolemic and hypovolemic piglets.

NORMOVOLEMIA and EPINEPHRINE

Effects of epinephrine on MAP (Figure 1)

MAP increased from baseline at all doses of epinephrine reaching significance ($p<0.05$) at 1.6 and 3.2 μg/kg/min.

Effects of epinephrine on SMABF (Figure 2)

SMABF decreased from baseline at all doses of epinephrine reaching significance only at high dose (3.2μg/kg/min).

Effects of epinephrine on SMAVR (Figure 3)

SMAVR increased at all doses reaching significance at 1.6 and 3.2 μg/kg/min.

Effects of epinephrine on RBF (Figure 4)

RBF decreased for all doses except 0.4 μg/kg/min where there was a small increase of 0.9%. A significant decrease ($p <0.05$) was seen only at 3.2μg/kg/min.

Effects of epinephrine on RVR (Figure 5)

RVR increased at all doses, but was significant only at 3.2μg/kg/min.

HYPOVOLEMIA and EPINEPHRINE

Effects of epinephrine on MAP (Figure 6)

MAP increased for all doses attaining significance at 1.6 and 3.2. μg/kg/min

Effects of epinephrine on SMABF (Figure 7)

SMABF increased at 0.2 and 0.4 μg/kg/min then progressively decreased with higher doses, reaching significance at 3.2. μg/kg/min.

Effects of epinephrine on SMAVR (Figure 8)

SMAVR decreased at 0.2 and 0.4 μg/kg/min then progressively increased, attaining significance only at 3.2 μg/kg/min.

Effects of epinephrine on RBF (Figure 9)

RBF increased at 0.2 μg/kg/min then decreased with higher doses, reaching significance only at 3.2. μg/kg/min.

Effects of epinephrine on RVR (Figure 10)

RVR decreased at 0.2 μg/kg/min then increased to reach significance at 3.2 μg/kg/min.

EFFECTS OF HYPOVOLEMIA, NO EPINEPHRINE

Effects of hypovolemia on MAP (Figure 11)

After an initial decrease in MAP with hypovolemia, the MAP rose above baseline at 50 minutes and then gradually decreased for the remaining 2.5 hours.

Effects of hypovolemia on SMABF (Figure 12)

After an initial decrease with hypovolemia, SMABF progressively increased to above baseline at 120 minutes and remained above baseline to the completion of the 3.5 hours.

Effects of hypovolemia on SMAVR (Figure 13)

SMAVR initially increased after hemorrhage but then decreased at 120 minutes to remain below baseline for the period of observation.

Effects of hypovolemia on RBF (Figure 14)

RBF initially decreased after hypovolemia but then increased at 120 minutes to remain above baseline at 3.5 hours.

Effects of hypovolemia on RVR (Figure 15)

RVR showed a more variable trend with an initial decrease below baseline, followed by a brief increase and subsequent fall below baseline over the latter period of observation.

Table 1

Baseline Values[a]

Piglet	Weight (kg)	MAP (mmHg)	RBF (ml/min)	RVR (PRU)	SMABF (ml/min)	SMAVR (PRU)	Na[b] (mmol/L)	K[b] (mmol/L)	Glc[b] (mg/dL)
1	1.6	100.76			51.3	1.96			
2	1.7	77.16	14.07	5.2	54.78	1.34	153	3.4	48
3	1.8	55.76	19.2	2.75	27.41	1.92	149	3.54	81
4	2.3	87.76	25.03	3.43			149.8	2.84	130
5	2.2	86.42	10.76	6.36	59.07	1.16	157.3	3.88	72
6	1.5	91.01	21.28	3.85	108.88	0.75	164.3	4.06	180
7	2.3	96.52			124.66	0.68	162.9	4.92	92
8	2	83.38	7.54	10	43.47	1.73	149.2	2.62	177
9	2	76.29			43.95	1.58	151.2	3.61	82
10	1.4	90.69			98.15	0.86	166	3.32	58
11	1.9	93.57	10.42	7.92			150.5	3.15	72
12	1.8	88.75			59.15	1.48	151.3	3.28	77
13	2.1	81.2			113.64	0.67			
14	2.4	83.24			104.86	0.71	148.9	2.45	98
15	2.5	73.87	33.38	2.06	87.72	0.79	151.4	3.89	
16	2.3	72.3	8.6	7.71	216.24	0.31	153	3.79	70
mean	2.0	83.67	16.7	5.48	85.23	1.14	154.1	3.48	95.2
s.d.[c]	0.33	11.0	8.7	2.7	48.74	0.53	6.0	0.64	42.0

[a] Values recorded for the initial ten minute baseline prior to receiving epinephrine.
[b] Sodium (Na), potassium (K), and glucose (Glc) serum levels drawn at time of induced hemorrhage.
[c] Standard deviation

Table 2

Absolute values[a]

			Normovolemia			
Epinephrine (ug/kg/min)	**MAP** (mmHg)	**SMABF** (ml/min)	**SMAVR** (PRU)	**RBF** (ml/min)	**RVR** (PRU)	**CVP** (mmHg)
baseline mean[b]	78.9 (10.3)	79.8 (43.6)	1.15 (0.52)	19.2 (10.6)	5.1 (2.97)	6.2 (3.7)
0.2	78.8 (13.8)	78.4 (43.8)	1.25 (0.91)	19.5 (12.1)	6.13 (5.32)	5.8 (3.7)
0.4	82 (13.1)	78.8 (47.8)	1.28 (0.87)	18.4 (10.2)	5.4 (3.8)	6.8 (4.2)
0.8	88.7 (16.5)	77.6 (46.1)	1.56 (1.28)	18.8 (10.9)	5.45 (3.42)	7.6 (4.5)
1.6	102.2 (20.1)	65.6 (52.9)	2.2 (1.41)	16.3 (9.74)	8.0 (6.17)	8.5 (4.1)
3.2	113.8 (23.4)	58.8 (46.5)	2.45 (1.38)	11.9 (8.23)	15.3 (13.1)	10.1 (4.3)
			Hypovolemia			
baseline initial[c]	65.2 (11.9)	66.8 (45.1)	1.53 (1.5)	14.9 (10.4)	6.57 (6.36)	4.2 (3.6)
baseline mean[b]	68.1 (10.6)	79.7 (44.7)	1.14 (0.73)	19.7 (12.4)	5.43 (5.56)	3.9 (3.1)
0.2	68.6 (12.2)	83.1 (45.5)	1.02 (0.63)	20.1 (14.4)	4.2 (2.1)	3.3 (3.1)
0.4	71.6 (11.7)	85.9 (46.3)	1.1 (1.0)	20.9 (13.6)	7.03 (10.4)	4.4 (3.5)
0.8	74.6 (11.7)	74 (50)	2.03 (3.5)	17.6 (12.6)	9.02 (14.1)	5.2 (3.7)
1.6	85.7 (14.6)	75.3 (56.4)	1.65 (1.04)	16.9 (11.2)	7.25 (6.71)	6.3 (3.6)
3.2	100.5 (20.7)	52.7 (43.5)	3.26 (2.45)	12.1 (11.4)	14.9 (12.0)	6.9 (3.4)

[a] expressed as mean (standard deviation)
[b] average baseline including initial baseline and baseline measured between epinephrine doses
[c] initial post-hemorrhage baseline

Table 3

Percentage change from baseline[a]

Normovolemic	EPI	MAP %Δ	SMABF %Δ	SMAVR %Δ	RBF %Δ	RVR %Δ
	0.2	2.7 (8.9)	-1.8 (11.1)	5.3 (13.7)	-1.7 (14.6)	12.5 (23.7)
	0.4	4.9 (10.8)	-1.8 (12)	9.2 (22.6)	0.9 (15.8)	4.5 (21.7)
	0.8	11.7 (17.5)	-2.7 (24)	23.7 (48.6)	-0.8 (25.4)	12.1 (35.8)
	1.6	31.5 (24.5)*	-9 (46.5)	73.5 (84.3)*	-9.6 (19.6)	55 (49.5)
	3.2	40 (20.5)*	-32.1 (33.8)*	147.3 (114.8)*	-43.2 (21.2)*	186.6 (150.2)*
Hypovolemic						
	0.2	2.2 (5.4)	7.3 (17.8)	-2.5 (15.9)	4.2 (15.8)	-2.7 (14)
	0.4	3.9 (4.6)	11.6 (19.6)	-4.5 (16.3)	-1.2 (8.4)	4.3 (9.5)
	0.8	8.9 (10.6)	-4.3 (26.5)	23.4 (49)	-1.6 (30.3)	17.1 (41.4)
	1.6	25.4 (11.5)*	-12.3 (27.9)	57.4 (54.2)	-19.5 (18.4)	58.2 (35.3)
* denotes p<0.05	3.2	51.2 (28.3)*	-34 (42.3)*	220.3 (177.2)*	-36.6 (31.7)*	211.6 (186)*

[a] percentage change from baseline for each epinephrine dose, EPI (epinephrine dose in μg/kg/min), %Δ (percentage change), values expressed as mean (standard deviation)

Figure 1

Effects of Epinephrine on Mean Arterial Pressure Normovolemia

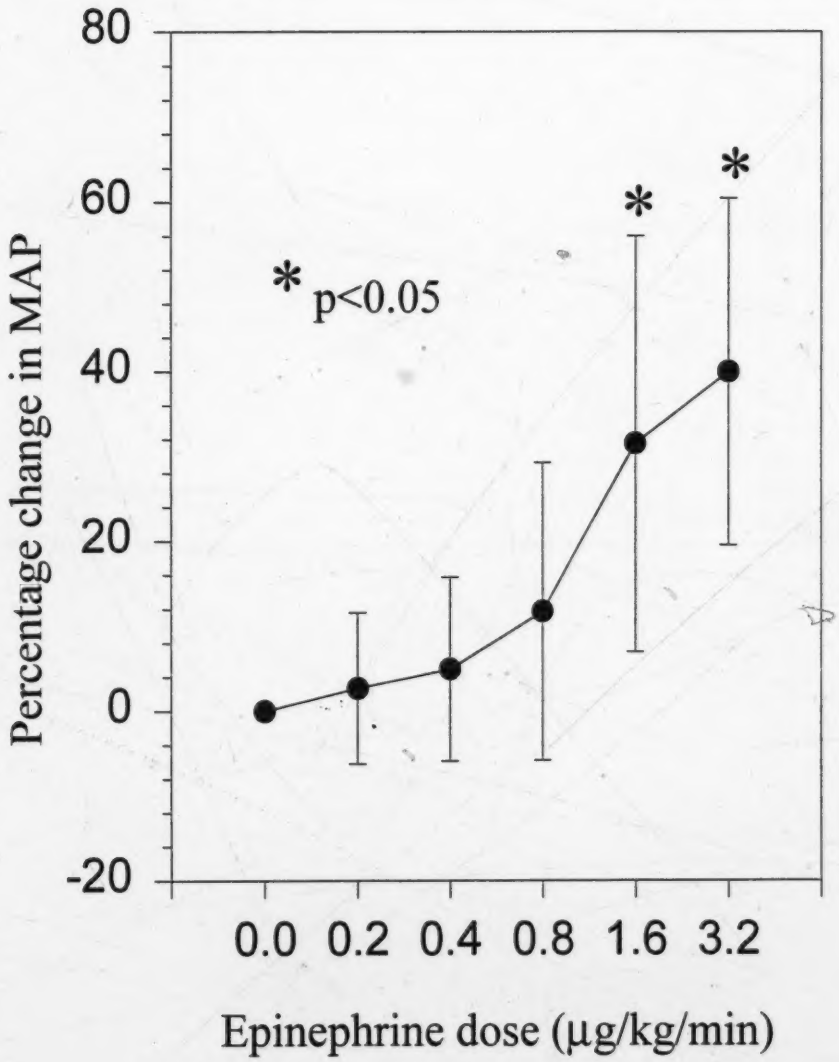


Figure 2

Effects of Epinephrine on Superior Mesenteric Artery Blood Flow Normovolemia

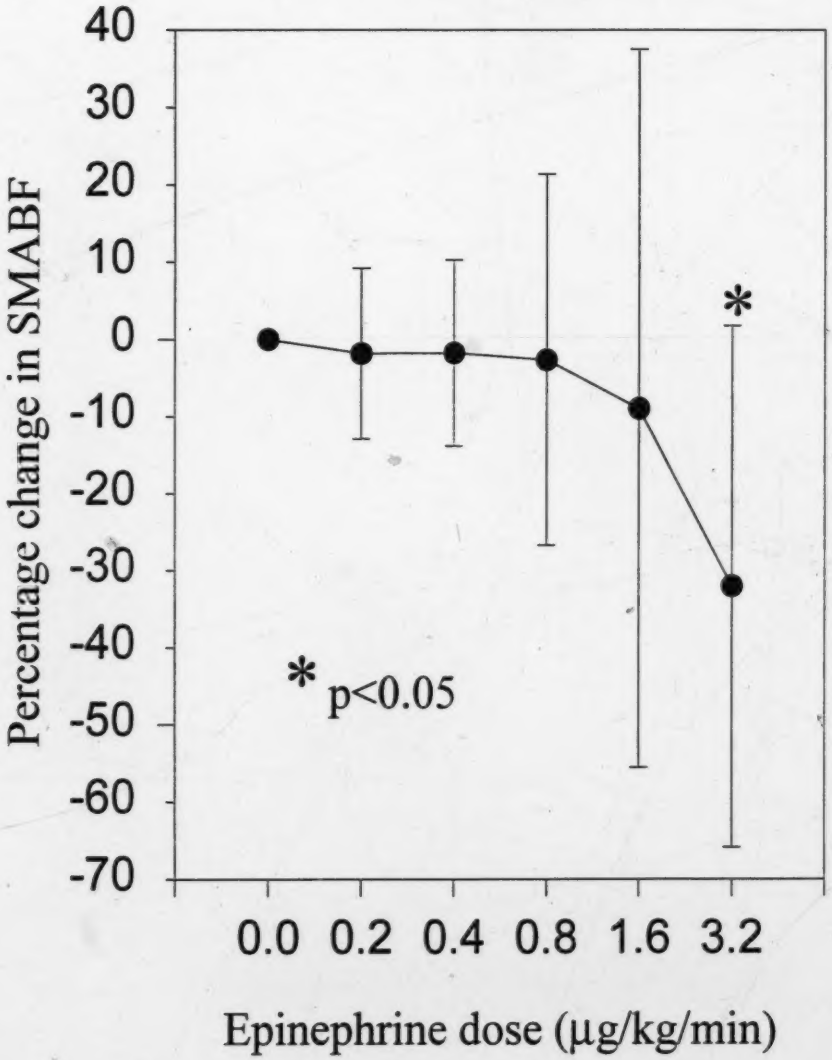


Figure 3

Effects of Epinephrine on Superior Mesenteric Artery Vascular Resistance Normovolemia

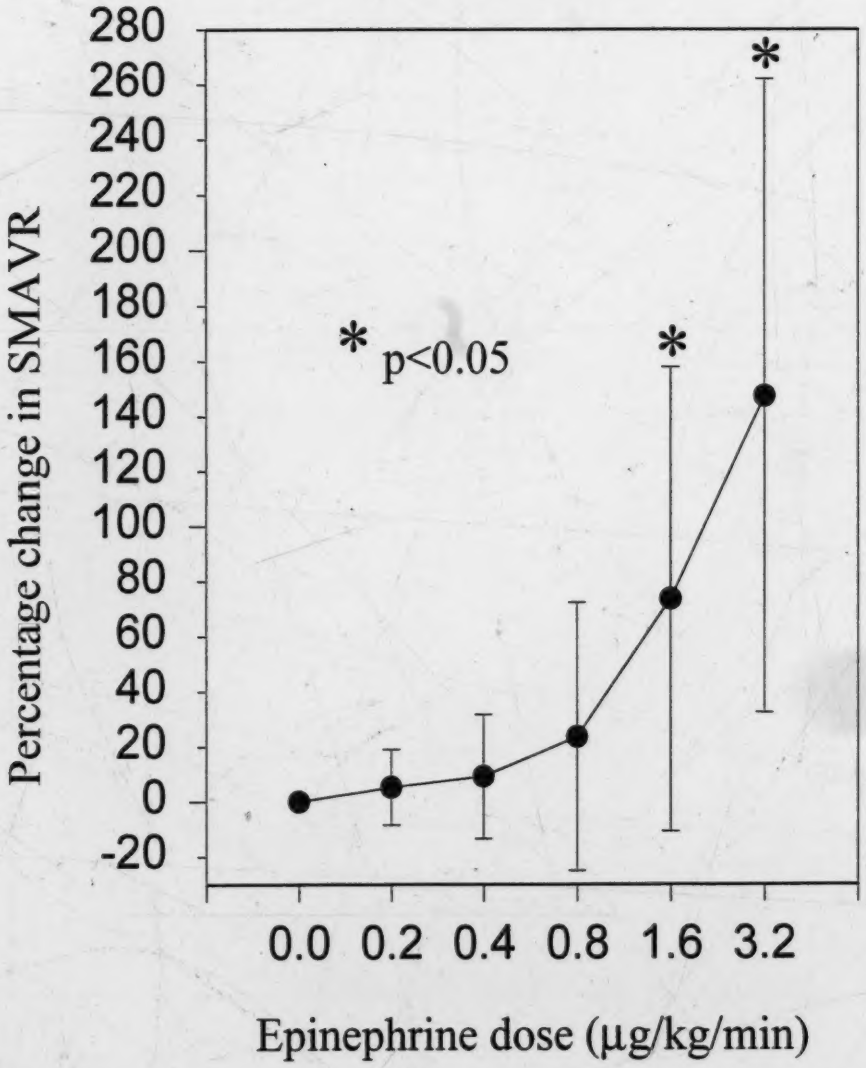


Figure 4

Effects of Epinephrine on Renal Blood Flow Normovolemia

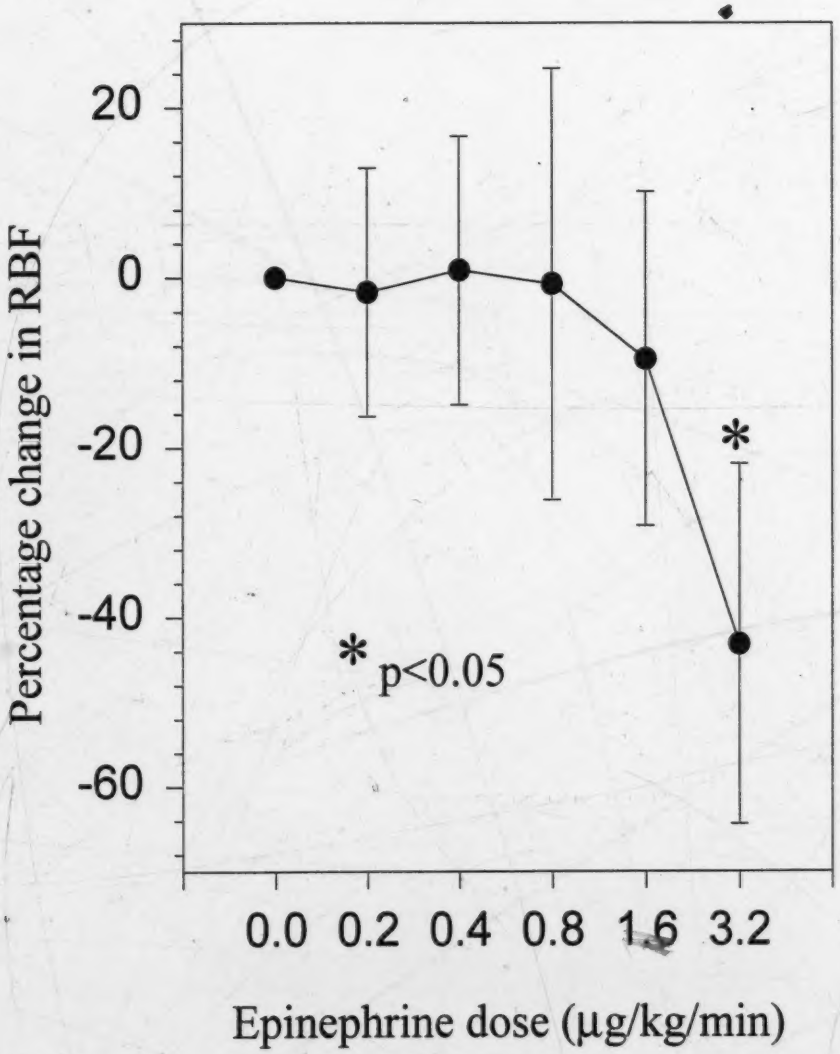


Figure 5

Effects of Epinephrine on Renal Vascular Resistance Normovolemia

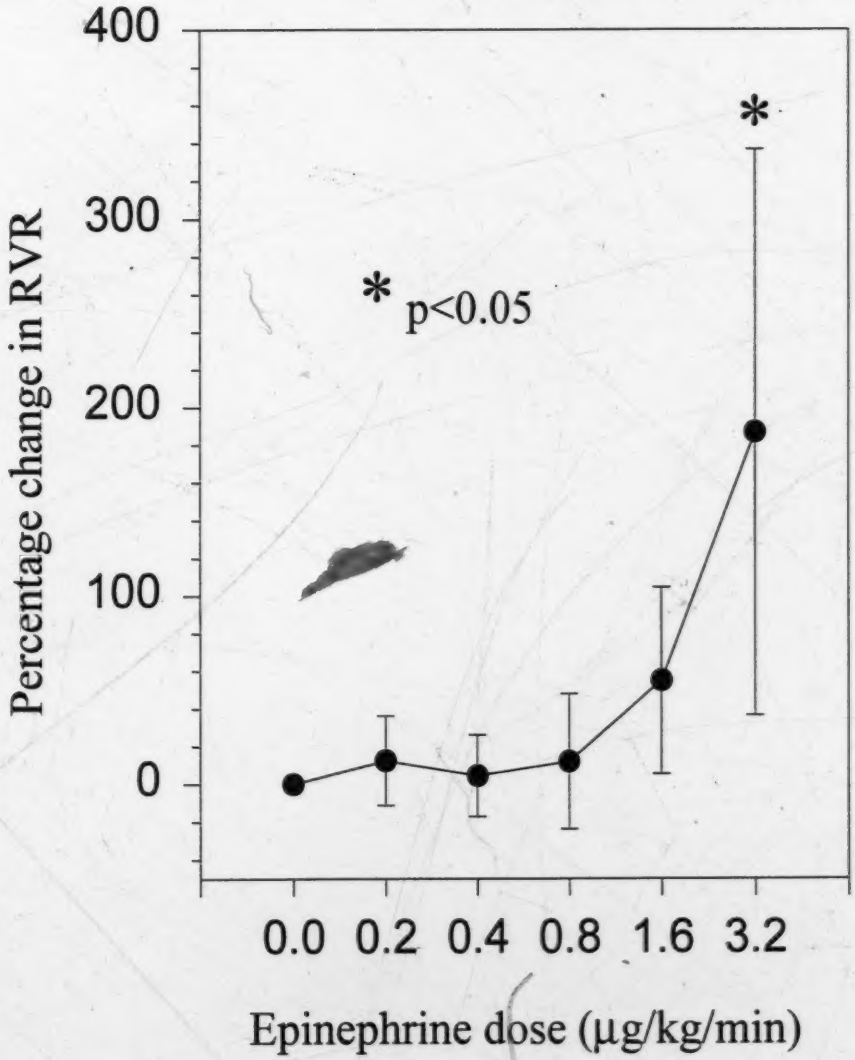


Figure 6

Effects of Epinephrine on Mean Arterial Pressure Hypovolemia

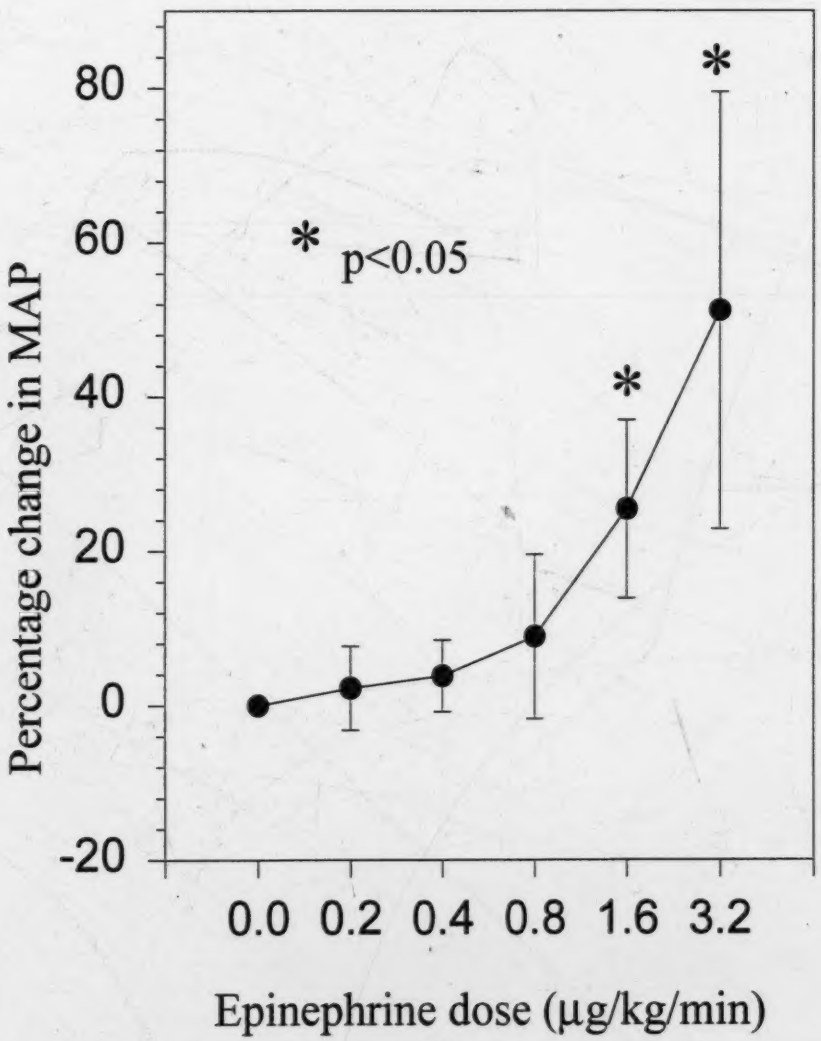


Figure 7

Effects of Epinephrine on Superior Mesenteric Artery Blood Flow Hypovolemia

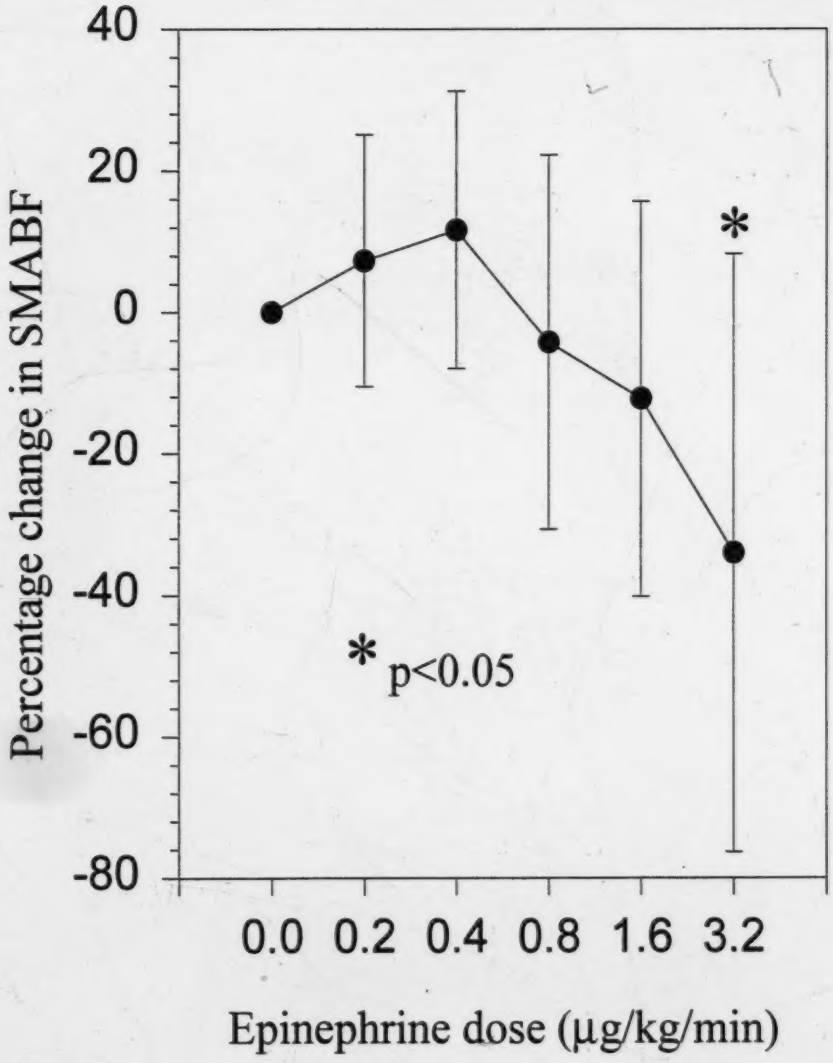


Figure 8

Effects of Epinephrine on Superior Mesenteric Artery Vascular Resistance Hypovolemia

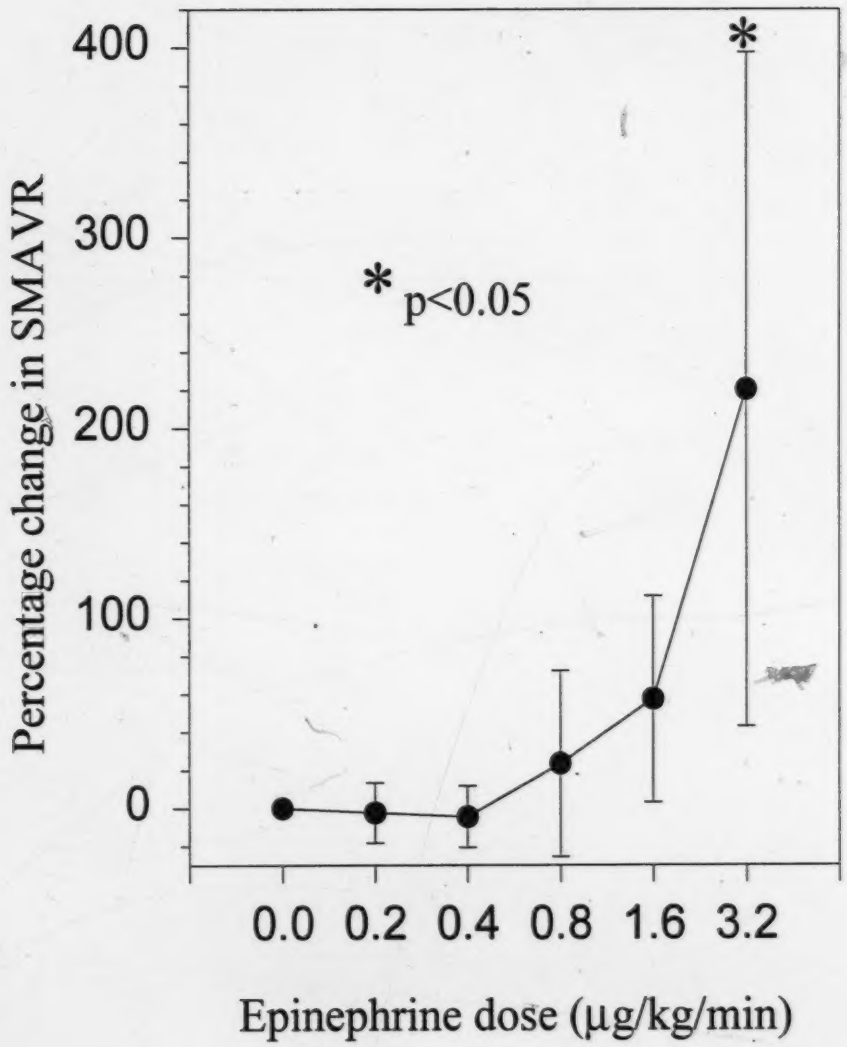


Figure 9

Effects of Epinephrine on Renal Blood Flow Hypovolemia

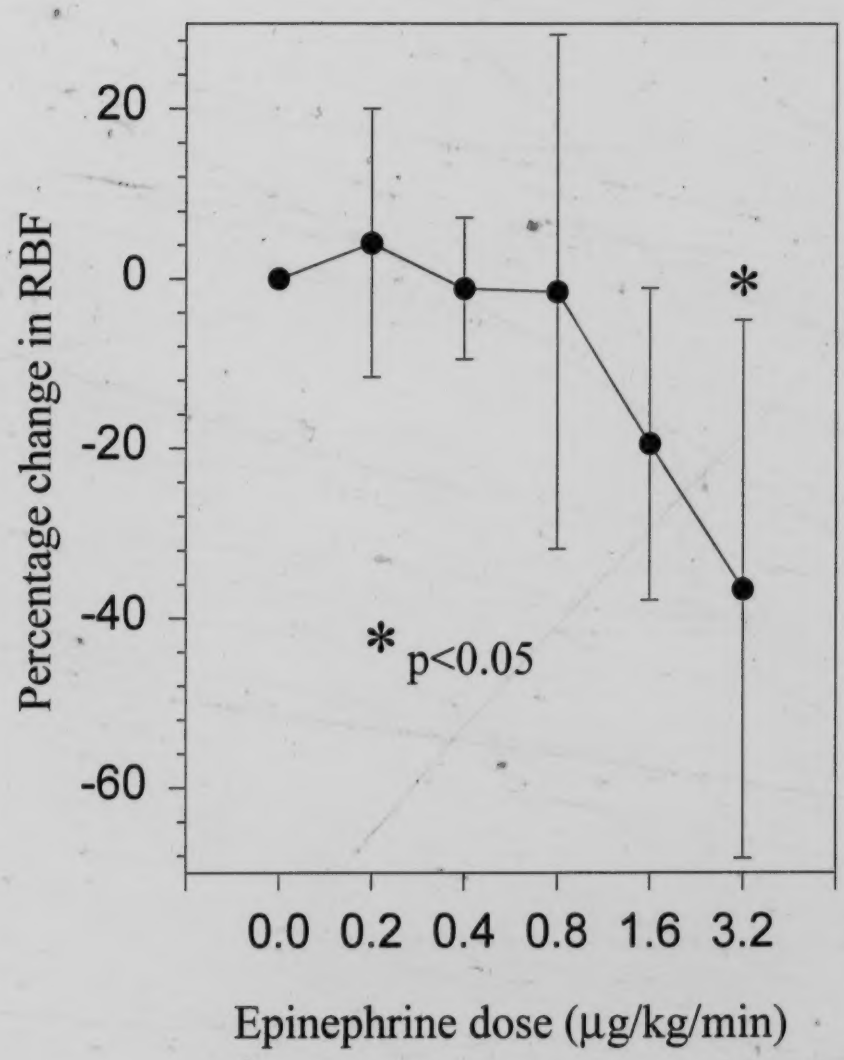


Figure 10

Effects of Epinephrine on Renal Vascular Resistance Hypovolemia

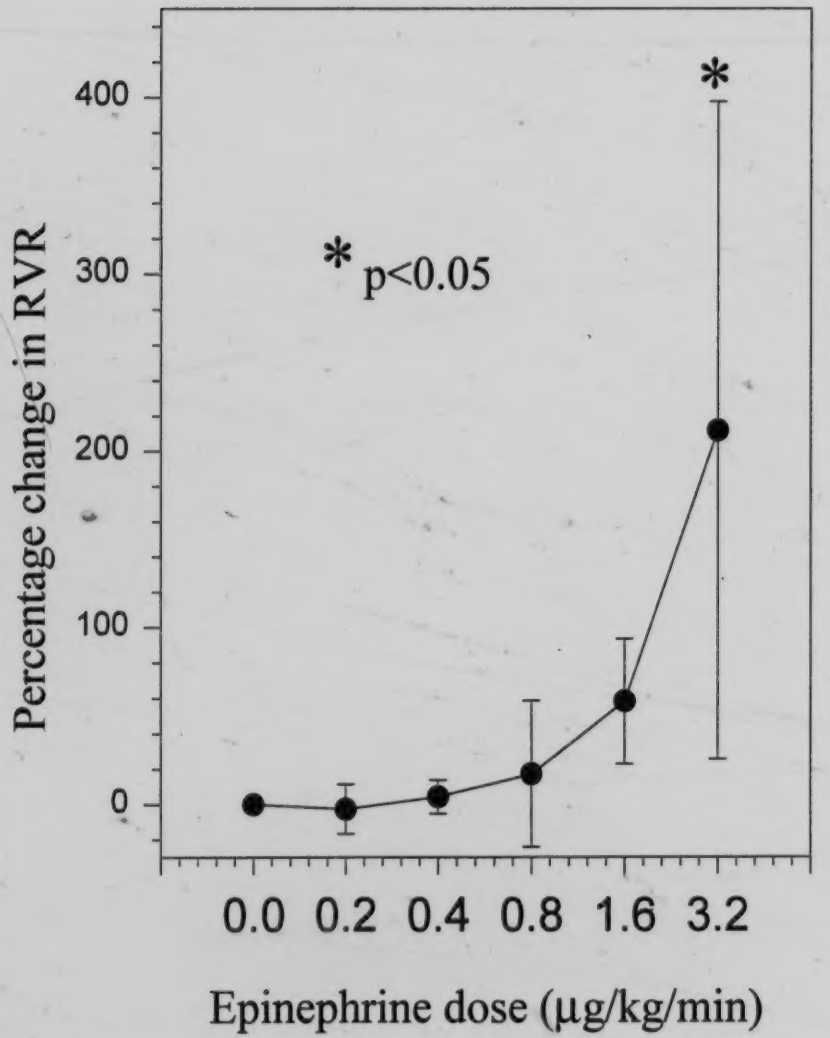
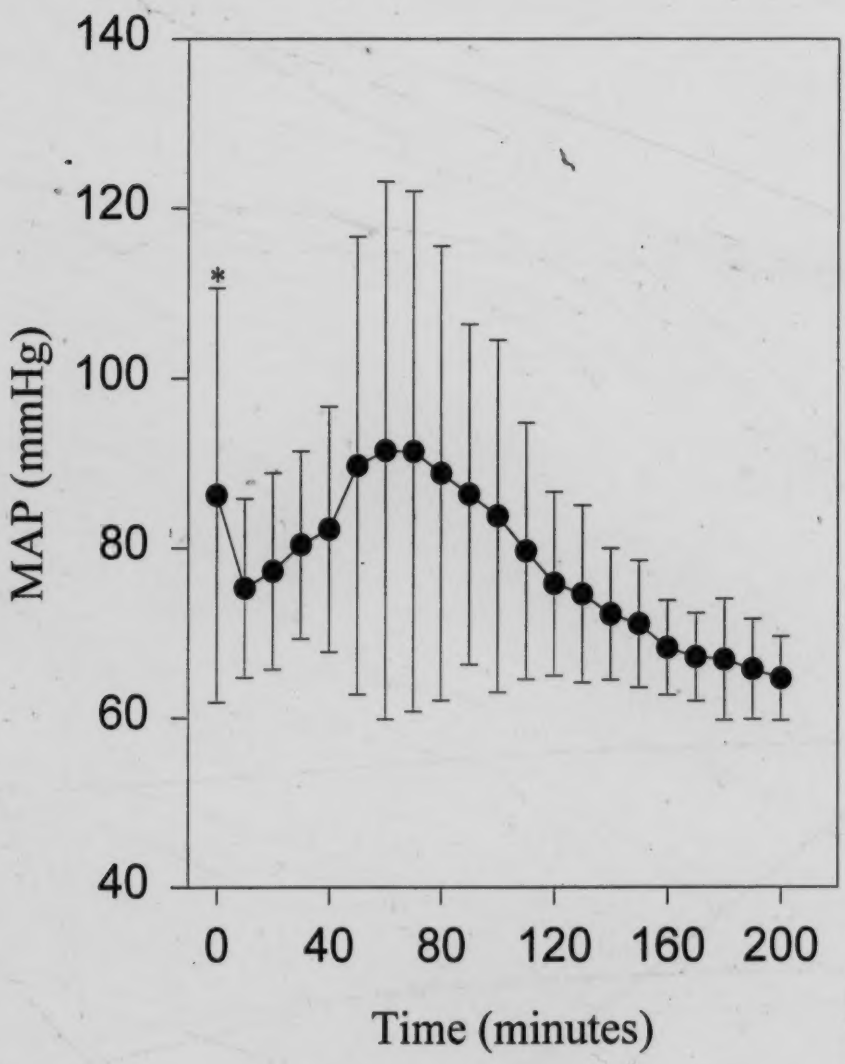


Figure 11

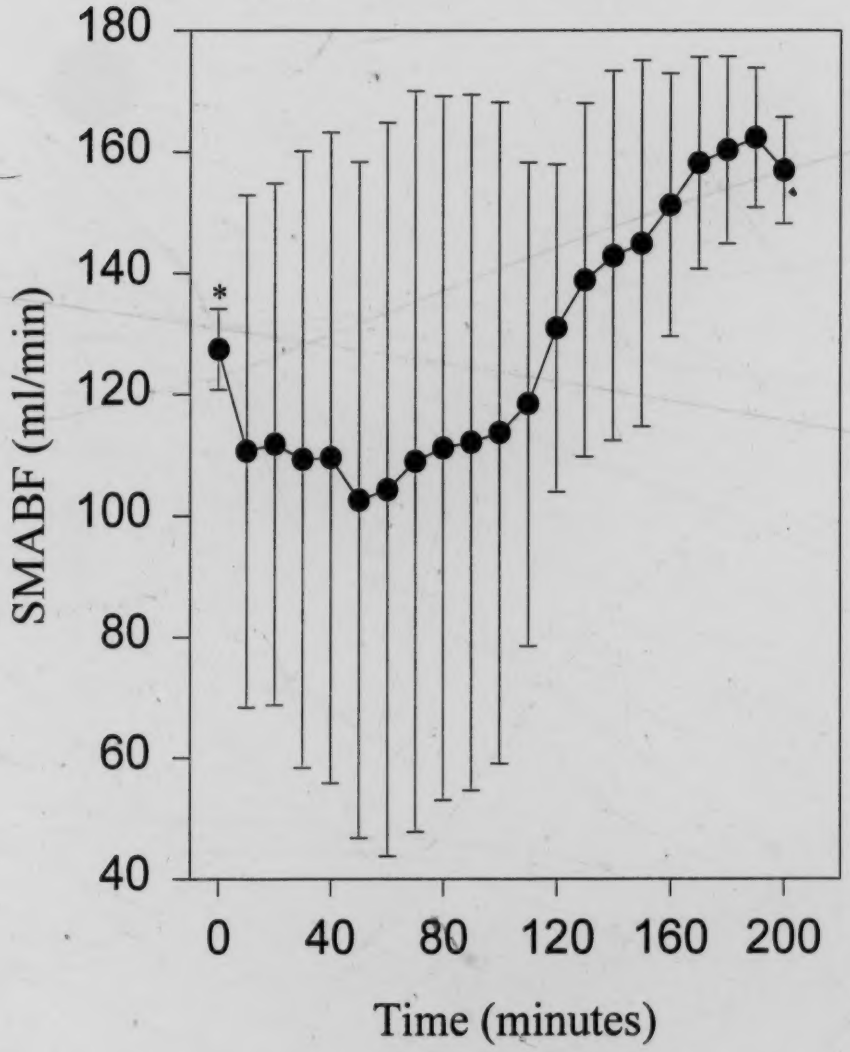
Effects of Hypovolemia on Mean Arterial Pressure



* baseline prior to hemorrhage

Figure 12

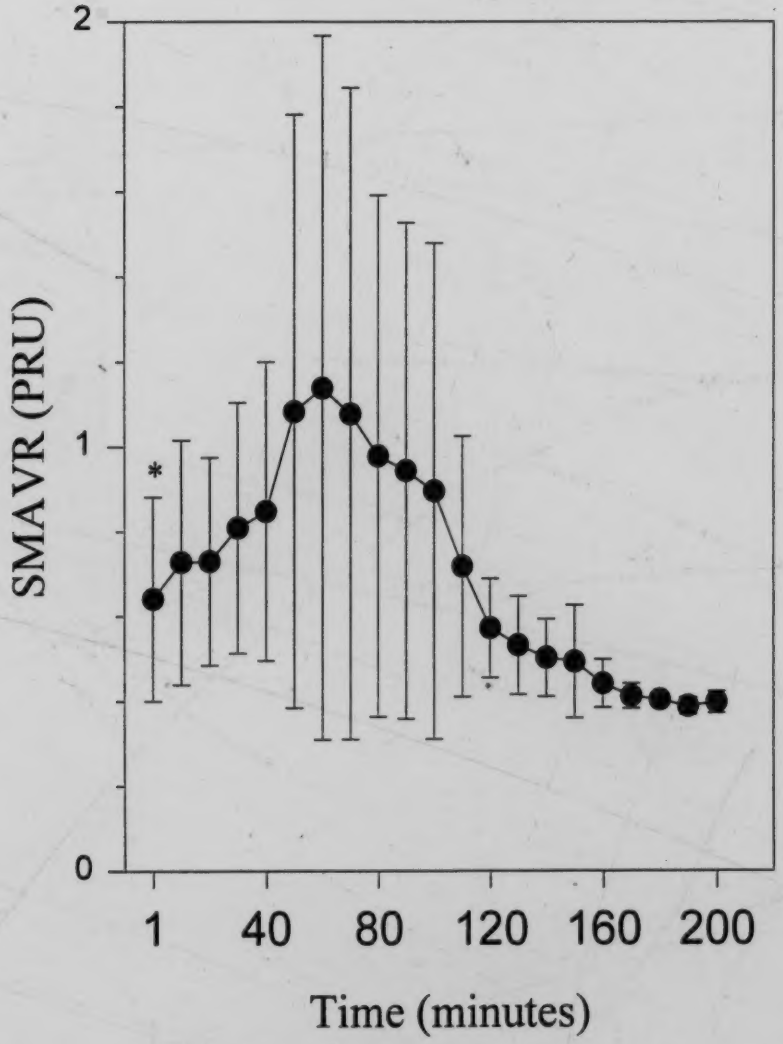
Effects of Hypovolemia on Superior Mesenteric Artery Blood Flow



* baseline prior to hemorrhage

Figure 13

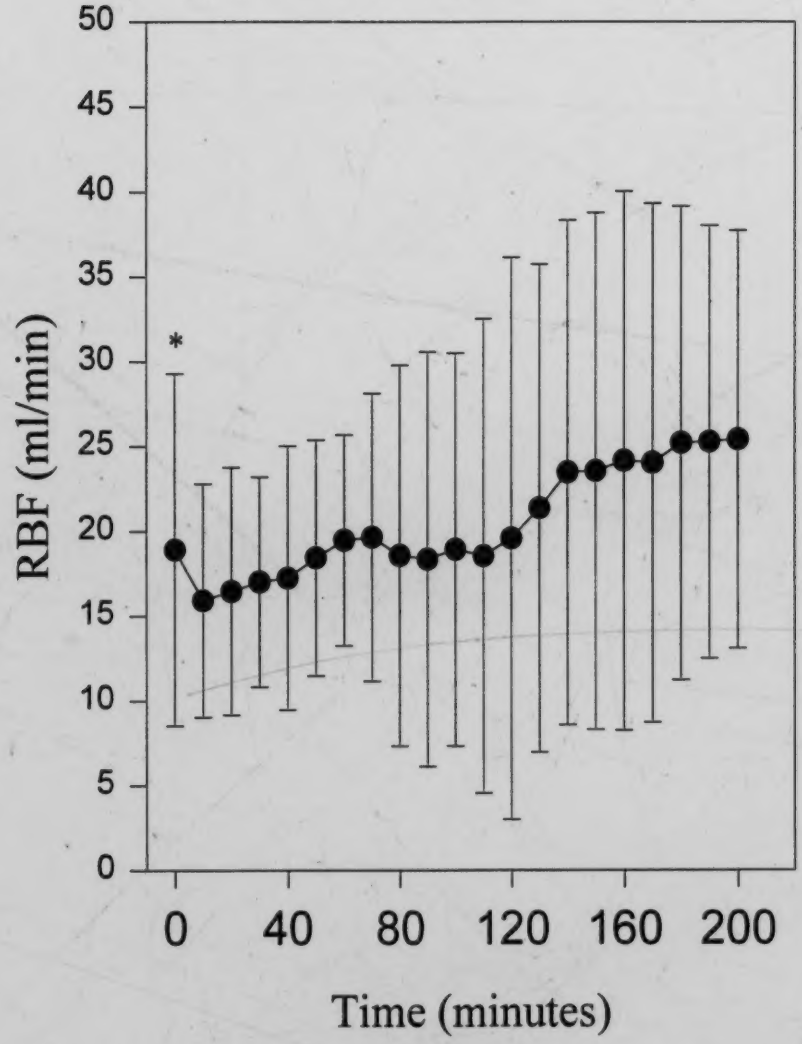
Effects of Hypovolemia on Superior Mesenteric Artery Vascular Resistance



* baseline prior to hemorrhage

Figure 14

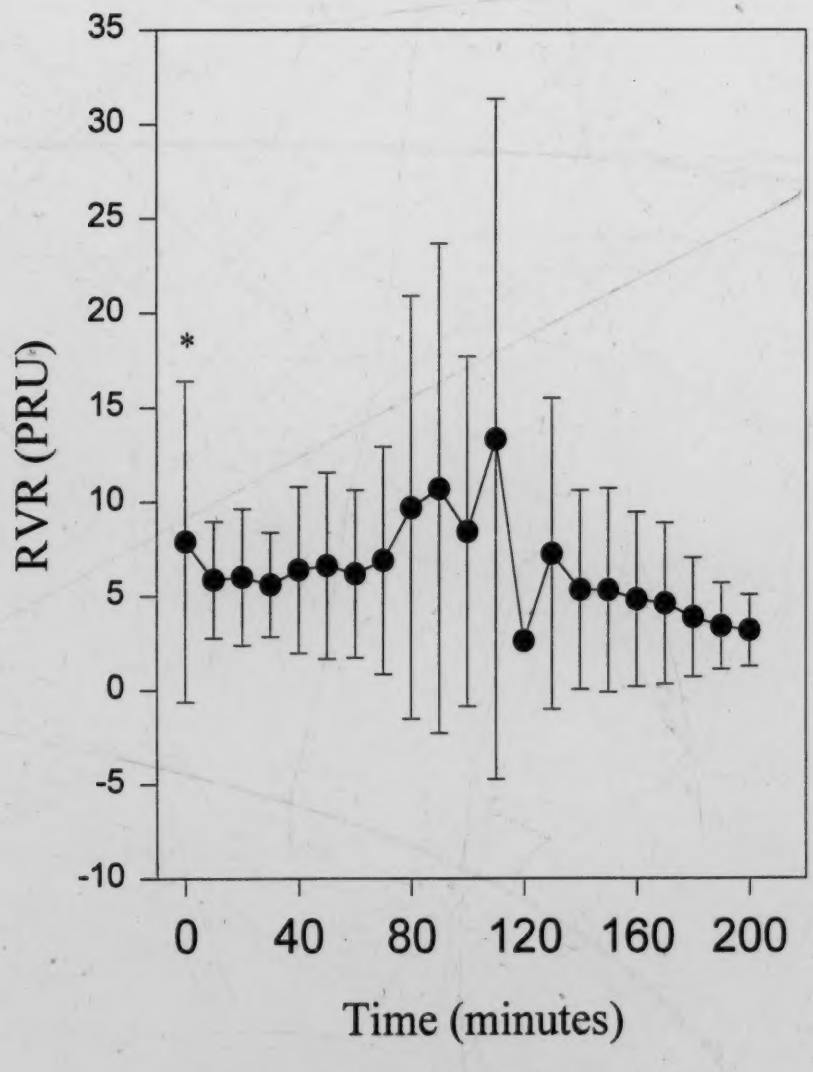
Effects of Hypovolemia on Renal Blood Flow



* baseline prior to hemorrhage

Figure 15

Effects of Hypovolemia on Renal Vascular Resistance



* baseline prior to hemorrhage

Chapter 10

Discussion

The use of epinephrine in newborns has been based on adult use with surprisingly little clinical or laboratory analysis to date. The search for an ideal inotrope in the neonate needs to be continued. Dopamine has traditionally been used as the first line inotrope however there is accumulating evidence that this drug is not as advantageous in the newborn as it is in the adult (14,52,80,107,108). Therefore other drugs, such as epinephrine, dobutamine and isoproterenol need further study. Epinephrine was chosen because of its potential for improving the status of a critically ill neonate, by improving variables such as CO, MAP and regional blood flow, and because of the paucity of previous studies on its effects on the newborn.

Of the studies performed previously, the majority have been acute experiments, using healthy animals and using bolus injection of exogenous catecholamines (31-37,64,65). While these studies have led to an increased understanding of the cardiovascular response in piglets, the clinical applicability may be questionable. The current study was performed using a continuous infusion of epinephrine, in a chronic model, in both normovolemic and hypovolemic piglets.

Epinephrine used as a bolus medication has been studied extensively with consistent results (86,113). However in the setting of the neonatal intensive care

unit, inotropes are commonly used as continuous infusions. The temporal effects of inotropes in the newborn is an area of study which appears to be rather important but has received very little study. The effect of epinephrine infusion on regional blood flow to the renal and mesenteric circulation in the newborn has not previously received attention.

The use of an acute model allows for more invasive monitoring, however the potential exists for the confounding effects of anesthesia on cardiovascular responses (5,10,15,25,88,132). A chronic model without the effects of either anesthesia or sedation should allow for direct study of the cardiovascular response to exogenous catecholamines.

Since there is little clinical use for inotropes in the non-stressed, normovolemic neonate, it was felt important to characterize the circulatory responses to epinephrine first in the normovolemic and then in the hypovolemic piglet. Hypovolemia in the critically ill neonate is quite common, and often inotropic support is required, however, very little work has been done in this area. Although there are several ways one may induce "shock" in an experimental model including sepsis, cardiogenic shock and hypovolemia, hypovolemia was selected as most promising to give consistent and reproducible data. Hypovolemia produces a hemodynamic profile similar to other forms of shock in the neonate with a decrease in CO and MAP and increase in SVR. A volume of 20 ml/kg of blood was withdrawn via the arterial line over 5 minutes, representing approximately 25% of each piglets blood volume (90).

The effect of epinephrine on MAP seen in this study is consistent with that seen previously (13), with epinephrine increasing MAP at all doses in both the normovolemic and hypovolemic groups including a significant ($p<0.05$) increase at 1.6 and 3.2 μg/kg/min. High dose epinephrine (3.2 μg/kg/min) increased MAP in the normovolemic and hypovolemic groups by 40 and 51.2% respectively (figures 1 and 6).

Low dose epinephrine (<0.2 μg/kg/min) has previously been shown to decrease MAP, likely via action on β_2-receptors (137). This was not seen in the current study, possibly because the lowest dose used was 0.2 μg/kg/min. The percentage change in MAP for each epinephrine dose was similar in both groups although the absolute value was lower in the hypovolemic group (table 2).

The increase in MAP was not statistically significant at low doses. However, because MAP is determined by CO and SVR, there could still be a clinically important increase in CO, and decrease in SVR, with relatively little change in MAP. Indeed, a significant increase in CO with a non-significant increase in MAP, at low dose epinephrine, has been seen in other studies (13,19,23,44).

Cardiac output is rarely measured directly (via Swan-Ganz catheter) in the human neonatal population because of volume overload concern if warm fluid is used or because of possible hypothermia if cold fluid is used. Therefore cardiac output is often inferred indirectly by measures such as MAP and urine output. However, the measurement of CO in a study similar to this might be helpful to

confirm that there is, in fact, an increase in CO despite minor changes in MAP with low dose epinephrine.

SMABF was decreased with higher epinephrine dose in the normovolemic group (figure 2). The percentage decrease from baseline was less than 3% for doses of 0.8 μg/kg/min and less, however, the increase in SMAVR showed a steady climb from 5% at 0.2 μg/kg/min to 23.7% at 0.8 μg/kg/min (figure 3). From this one can say that SMABF is fairly well maintained despite an increase in SMAVR for low to moderate dose epinephrine. This is due to the elevation in MAP with increasing epinephrine dose. At high dose epinephrine (3.2 μg/kg/min) the decrease in SMABF of 32.1% was significant ($p<0.05$) with a markedly elevated SMAVR (147.3% above baseline).

In contrast to the normovolemic group, in hypovolemic piglets there was an increase in SMABF from baseline of 7.3% and 11.6% for epinephrine doses of 0.2 and 0.4 μg/kg/min respectively (figure 7). This increase in flow was accompanied by a decrease in SMAVR of 2.5% and 4.5% for the same doses (figure 8). As in the normovolemic group there was a mild (4.3%) decrease in SMABF at 0.8 μg/kg/min with a SMAVR 23.4% above baseline. At 3.2 μg/kg/min of epinephrine, there was a significant decrease in SMABF (34%, $p<0.05$) and increase in SMAVR (220.3%, $p<0.05$). This group reveals even stronger evidence that SMABF can be maintained (and perhaps increased) with low to moderate dose epinephrine.

The renal circulatory response to epinephrine infusion was similar to the mesenteric response. In normovolemic piglets, the decrease in RBF was less than

2% for doses of 0.8 μg/kg/min and less, in fact RBF increased 0.9% at 0.4μg/kg/min, while the increase in RVR was 12.5% or less for doses up to 0.8 μg/kg/min (figures 4 and 5). This demonstrates that RBF is maintained at doses as high as 0.8 μg/kg/min despite an increase in RVR. At high dose epinephrine (3.2μg/kg/min) RBF decreased and RVR increased significantly ($p<0.05$).

Hypovolemic piglets demonstrated an average increase in RBF of 4.2% with a 2.7% decrease in RVR from baseline at 0.2 μg/kg/min of epinephrine (figures 9 and 10). There was a mild decrease in RBF of 1.2% and 1.6% at 0.4 and 0.8 μg/kg/min respectively with RVR increasing up to 17.1% at 0.8 μg/kg/min. Once again this demonstrates that RBF is maintained with low to moderate dose epinephrine. High dose epinephrine (3.2μg/kg/min) caused a 36.6% decrease in RBF from baseline with a 211.6% increase in RVR, both of which were statistically significant. Low to moderate dose epinephrine should not lead to the development of mesenteric or renal ischemia as there is no accompanying significant decrease in SMA or renal blood flow. In fact, there was a trend toward improved SMA and renal blood flow in the hypovolemic piglet when low dose epinephrine was infused. When this is coupled with the advantageous effects of increased MAP and CO, the benefits of epinephrine to the hypotensive neonate may have important clinical implications.

High dose epinephrine, on the other hand, leads to significant decreases in SMA and renal blood flow which may indeed lead to mesenteric or renal ischemia. It should be emphasized however, that the effect of long term (eg. several days)

epinephrine infusion in the neonate has yet to be determined. There may be other factors such as autoregulatory escape (see below) that attenuate this vasoconstriction.

Hypovolemia led to an average decrease of 13.7 mmHg (17.4%) in the initial post-hemorrhage baseline MAP (table 2). This decrease in MAP is likely associated with a decrease in CO and increase in SVR (18). Hypovolemia caused a 13 ml/min and 4.3 ml/min (16% and 22.4%) decrease in SMA and renal blood flow respectively. This was accompanied by a 0.38 PRU and 1.47 PRU (33% and 28.8%) increase in SMA and renal vascular resistance. These post-hemorrhage baseline values are consistent with previous studies (54,65) showing increased vascular resistance and decreased blood flow to the mesenteric and renal circulations. As time passed during monitoring, the subsequent baseline values between epinephrine doses did not return to these original hypovolemia baseline values, therefore the percentage change from the immediately preceding baseline for each epinephrine dose was analyzed.

Variable baseline blood flow parameters post-hemorrhage were further investigated in a second group of piglets subjected to hypovolemia (bled 20 ml/kg) but which did not receive epinephrine. In this further group of 5 piglets, baseline MAP decreased post-hemorrhage, transiently increased above baseline, and finally fell below the original baseline (figure 11). SMABF decreased post-hemorrhage but increased to supra-baseline levels 2 hours post-hemorrhage and stayed above baseline for the remaining 3.5 hour period of observation (figure 12). SMAVR

increased post-hemorrhage, fell below baseline at 120 minutes and continued to decrease for the remainder of the experiment (figure 13). RBF and RVR (figures 14 and 15) were similar in three piglets studied. The temporal responses to hypovolemia were consistent with the data from the main study group.

The response to hemorrhage with an initial decrease in regional blood flow and MAP, found in the current study, was consistent with previous studies (7,54,65,111). In addition, the present study revealed an increase in MAP back to baseline levels at 50 minutes, with SMABF and RBF returning to baseline 120 minutes post-hemorrhage. This temporal response is similar to that seen by Bellamy et al in their study of organ blood flow after hemorrhage (18). Immature swine (20-25 kg) underwent a 41 ml/kg hemorrhage. There was an initial decrease in blood flow to the small bowel, but at 120 minutes post-hemorrhage, blood flow had increased above baseline with a decrease in vascular resistance. Immediately post-hemorrhage, blood flow to the renal cortex decreased significantly, followed by a gradual increase in RBF. This delayed increase in RBF did not, however, attain pre-hemorrhage levels by two hours despite a decrease in RVR.

Legal studied the effect of 33% blood volume acute hemorrhage in unanesthetized newborn piglets and found that MAP did not return to pre-hemorrhage levels until 24 hours post-hemorrhage (84). Dyess followed the redistribution of blood flow after 25% acute hemorrhage in piglets and observed that blood flow to the kidneys and bowel fell from baseline levels with little or no recovery until resuscitation began 60 minutes post-hemorrhage (54). Hanon studied

the effects of hemorrhage in 20-25 kg conscious pigs and found that MAP returned to control values after 5 hours of observation in pigs bled 30% blood volume but did not in those bled 50% blood volume (70). The differences in effects between the studies could be caused by piglet age, extent of hypovolemia induced, rate and site of hemorrhage and the time period monitored.

The differential response to epinephrine in normovolemic and hypovolemic piglets, in regard to mesenteric and renal vascular resistance and blood flow, as well as the spontaneous improvement in SMA and renal blood flow post-hemorrhage warrant further discussion.

A differential response to low dose epinephrine was detected between the normovolemic and hypovolemic groups, with a decrease in SMAVR and RVR and an increase in SMABF and RBF in the hypovolemic group. While these differences were not statistically significant, they raise questions regarding the specific responses to epinephrine in the stressed, hypovolemic piglet. Several explanations could be tendered to account for these differential responses, including changes in adrenergic-receptors, autoregulatory escape and reactive hyperemia.

In the stressed piglet, high endogenous catecholamine levels may lead to adrenergic-receptor desensitization. Desensitization is the process whereby the response to receptor stimulation becomes less with persistent or repeated stimulation. It may be due to a decrease in receptor number, binding affinity or in the coupling of the receptor binding to subsequent activation of a metabolic response (71,83). In the setting of high concentrations of epinephrine there may be

desensitization of either alpha- or beta-receptors. There is a fairly large amount of literature on beta-adrenergic receptor desensitization (71) but only minimal information on alpha-adrenergic receptor regulation (78). There is however very little known on either alpha- or beta-receptor regulation in the peripheral vasculature. With exposure to high levels of endogenous or exogenous catecholamines, both alpha- and beta-receptors may undergo desensitization. The exact time period required for desensitization to occur is yet to be delineated in vivo. In general however, receptor desensitization likely occurs by at least two mechanisms. The first is a change in the second messenger response to bound catecholamines, a change which could occur within minutes. A second mechanism is a decrease in receptor number or affinity, an effect which likely occurs over several hours (38,71,73,89,97,114,122,137).

There are high endogenous catecholamine levels in the stressed piglet, including epinephrine and norepinephrine (69). These high levels could lead, in turn, to a decreased adrenergic-receptor response by rapidly changing the second messenger system. It is possible that alpha-receptor desensitization may predominate over beta-receptor desensitization. A decreased alpha effect would then be seen when exogenous low dose epinephrine is infused with a consequent increase in apparent beta effect, yielding decreased mesenteric and renal vascular resistance and increased blood flow. This is a possible mechanism for the increased SMA and renal blood flow observed with low dose epinephrine in hypovolemic piglets in the present study.

As the exogenous epinephrine dose is increased, an increased alpha effect is observed. This increased alpha effect with high dose epinephrine could be related to receptor sensitivity, with higher dose epinephrine causing more alpha-receptor activation or a higher level of activation. Harden has proposed a similar mechanism for the beta-receptor. He states that if desensitization has occurred, the efficacy of agonists to increase intracellular cAMP will be reduced, therefore a higher concentration of hormone will be required to elicit the same biologic response (71).

It is apparent from previous studies (38,71,73,89,97) that both alpha- and beta-adrenergic receptor desensitization could have occurred in the time period used in this study. A study designed to directly compare alpha- and beta-receptor desensitization in a temporal fashion would help define the role of desensitization to epinephrine in peripheral vasculature.

Autoregulation is the ability of a vascular bed to maintain constant blood flow despite changes in perfusion pressure. In a hypertensive state the vascular bed responds by elevating vascular resistance to keep blood flow constant. In a hypotensive state the vasculature must respond by decreasing resistance to keep flow constant. Autoregulation may occur secondary to local tissue factors. Local control of blood flow may, in turn, be explained by the metabolic theory, where flow is determined by the metabolic needs of the tissue, such as oxygen, or by the myogenic theory where a sudden stretch of the arterioles causes a reflex increase in vascular resistance (68). In the metabolic control of local blood flow, the release of vasodilators, including adenosine, histamine and prostaglandins, during ischemia

may predominate over sympathetic vasoconstriction, returning blood flow to baseline values. Autoregulation has been found to be age-dependent in piglets with no significant autoregulation in the renal and mesenteric circulations until one and four weeks of age respectively (32,105).

In the current study, MAP increased with higher epinephrine dose. The resultant elevation in SMA and renal vascular resistance could be secondary to autoregulation (increased resistance to keep blood flow constant despite increased perfusion pressure) and to direct alpha stimulation. As the epinephrine dose is increased the alpha effect predominates leading to a decrease in blood flow and loss of autoregulation. However, after induction of hypovolemia, SMA and renal blood flows were decreased initially followed by gradual return to baseline. This suggests a lack of immediate autoregulation in these young piglets and is consistent with previous studies (32,105).

Autoregulatory escape is a term used to describe the "escape" from vasoconstriction caused by sympathetic stimulation, thereby allowing a return toward baseline blood flow. Mesenteric autoregulatory escape has been observed in piglets by one week of age (37,104). Bersten demonstrated renal autoregulatory escape in adult sheep during epinephrine infusion (23). Chen found that escape from sympathetic vasoconstriction occurs, in part, because a lower pH inhibits alpha-2 mediated postjunctional responses to neuronally released norepinephrine (41). This lower pH could occur after sympathetic stimulation caused a reduction in

blood flow that either diminished hydrogen ion washout or shifted metabolism to anaerobic pathways.

With the stress of hypovolemia there is an increased sympathetic discharge, with subsequent vasoconstriction of the mesenteric and renal arterioles, however recovery in blood flow with time was observed in this study which may be related to autoregulatory escape. Measuring plasma catecholamine levels may help to identify the presence of autoregulatory escape: if there is an improvement in blood flow, despite persistent elevation of plasma catecholamine levels, autoregulatory escape would be confirmed.

Reactive hyperemia is an example of metabolic control of local blood flow. That is, mechanisms that control blood flow in response to the metabolic needs of the tissues. When the blood supply to a tissue is occluded and then later restored, the tissue blood flow increases dramatically, often to about five times normal, and for a time period that is proportional to the time of occlusion. The released local factors that cause vasodilation, such as adenosine, work to repay the accrued oxygen deficit (68).

Reactive hyperemia is a likely explanation for the SMA and renal blood flow response to hypovolemia in this study. After an initial decrease in blood flow with induction of hypovolemia the baseline SMABF and RBF gradually increased over time. Presumably the above mentioned vasodilatory factors were released to repay the tissue metabolic debt. The reactive hyperemia response has been noted to be present at birth in piglets (32,37,106).

The differential response to low dose epinephrine seen after hemorrhage may also be related to reactive hyperemia. In a milieu of released vasodilators, alpha-receptor stimulation with low dose epinephrine may be ineffective, while the beta effect may be additive, leading to a decrease in SMA and renal vascular resistance in the hypovolemic piglets. At higher dose epinephrine, the alpha effect remained strong enough to overcome the local vasodilatory factors.

The mechanism involved in the differential response to epinephrine in normovolemic and hypovolemic piglets and the recovery in SMA and renal blood flow after hemorrhage may be multifactorial. Changes in adrenergic-receptor regulation, and the time course involved, can be investigated using radiolabeled adrenergic-receptor agonists and antagonists (83). Autoregulatory escape and reactive hyperemia may both be involved as well. Measuring plasma catecholamine levels over a given time period may help differentiate which of these two mechanisms is prominent. These are important areas of study as they may all be involved in altering the regional blood flow response to catecholamines. The answers obtained may prove to be particularly important in the critically ill neonate who requires inotropic support for several days.

This study examined the macrovascular effects of epinephrine on normovolemic and hypovolemic piglets. The microvascular effects may be quite different. Giraud studied the effects of both epinephrine and dopamine in anesthetized adult dogs (63). Vasodilator infusions of these inotropes decreased the delivery of oxygen to the intestinal tissue despite increasing total intestinal blood

flow. Techniques such as small bowel tonometry and inulin clearance could be employed to assess the effects of catecholamines on the microcirculation, as indirect measures of intestinal mucosal blood flow and glomerular filtration rate. In addition, further studies on oxygen delivery across different regional vascular beds comparing the effects of different inotropes may be very useful.

The critically ill neonate may require inotropic infusion for several days. The temporal response to epinephrine infusion has received little study in the neonate. If recovery in blood flow during epinephrine infusion occurs in neonates as seen in adult sheep (23), the risks of decreased SMA and renal blood flow with high dose epinephrine may be attenuated over time. The temporal response to just hypovolemia (no epinephrine) revealed interesting results despite the small number of piglets. In a subsequent larger study it would be interesting to do the same hypovolemic model but to also gather more information as to the underlying mechanisms. Such information as serum electrolytes, glucose, hematocrit, catecholamine and renin levels would be useful.

This study lends support to using low to moderate dose epinephrine in the hypotensive, critically ill neonate as probably the most important downfall of epinephrine historically has been a presumed marked decrease in renal and mesenteric blood flow which was not observed in this study. When combined with the advantageous effect of epinephrine on cardiac output and on systemic to pulmonary vascular resistance ratio for the neonate with persistent pulmonary

hypertension (13,14), epinephrine may well be a better alternative to dopamine in selected patients.

Chapter 11

Conclusion

This study lends support to using epinephrine in the hypotensive, critically ill neonate. Low to moderate dose epinephrine did not lead to a significant decrease in renal or mesenteric blood flow in either the normovolemic or hypovolemic piglet. In addition, these dosages of epinephrine are associated with beneficial increases in MAP and CO as seen in this, and previous, studies (13,14).

Although dopamine has long been considered to have a selective renal vasodilatory effect in low doses, this may not be true in neonates (107,108). When combined with the advantageous effect of epinephrine, compared to dopamine, on CO and on systemic to pulmonary vascular resistance ratio for the neonate with persistent pulmonary hypertension (13,14), epinephrine may well be a better alternative to dopamine in selected patients.

There is need for further study. Epinephrine may be effective at lower dosages than those used in this study, with an increased beta-adrenoceptor response and subsequent vasodilation. The temporal response to epinephrine in the neonate using longer infusion times in a chronic model warrants further characterization, as does the differential regional blood flow effects of epinephrine. Comparative data for different inotropes such as epinephrine, norepinephrine, dopamine and

dobutamine and evaluation in a chronic model incorporating clinical stress or hypovolemia would only add to the merit of further work.

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